

VOL. 41, PART 2

JUNE 1958

The British Mycological Society

(Recognosce notum, ignotum inspicere)

TRANSACTIONS

Edited by

G. C. AINSWORTH and S. D. GARRETT



CAMBRIDGE UNIVERSITY PRESS

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Trans. Brit. mycol. Soc. 41 (2), 145-156 (1958).

AIR SPORA OF AN ESTUARY

BY P. H. GREGORY AND T. SREERAMULU*

*Department of Botany, Imperial College of Science and Technology,
London, S.W. 7*

(With 4 Text-figures)

The spore content of air of an estuarine area has been studied for the first time by use of the Hirst automatic volumetric suction trap on Thorney Island, Chichester Harbour, during the first half of July 1954. Spores of some crop pathogens, including *Ustilago*, *Erysiphe* and *Alternaria*, were much less abundant than in agricultural areas, but diurnal changes in the air spora were in general similar in both areas. The dominant constituents of the air spora were basidiospores, *Cladosporium* and pollens. Concentrations of 'hyaline' basidiospores, mainly *Sporobolomyces* with their maxima in the early hours of the morning, were very much higher than those hitherto recorded. High humidity and low windspeed favoured high concentrations of this group which reached a maximum of a million per m.³. *Tilletiopsis*, the other important constituent of this group, was more sensitive to changes of weather than was *Sporobolomyces*. Large hyaline basidiospores occurred in appreciable numbers only after rainfall. The 'red-brown' and 'yellow' groups of basidiospores were almost equally abundant, and the effects of weather on their abundance was complex.

The occurrence of a blue-green alga, *Gloeocapsa*, in relatively high concentrations during the whole period of observation suggests that air dispersal may be important in some epiphytic algae. Its occurrence is of interest as a potential respiratory allergen.

The need for a continuous measurement of changes in composition of the air spora has long been recognized by plant pathologists and students of allergy, but it remained practically impossible until the development by Hirst (1952) of his automatic volumetric spore trap with which it is possible to follow the changes in the spore content of the air, both quantitatively and qualitatively, continuously over a long period, and the changes observed can be correlated with the changes in weather.

Existing knowledge of the spore content of the air comes mainly from inland agricultural and industrial areas (Durham, 1937, 1938; Ainsworth, 1952; Hirst, 1953; Gregory & Hirst, 1957); the air over oceans (Meier, 1935; Bisby, 1935; Rittenberg, 1939; Polunin, 1951; Pady & Kelly, 1953*a, b*, 1954); and on commercial air routes (Newman, 1948). In the present investigation the Hirst trap has been used to study the air spora in an estuarine area where the ecological factors are different from areas studied previously.

METHODS

A Hirst automatic volumetric spore trap was operated continuously at the Royal Air Force Station, in Thorney Island, Hants, from 30 June to 13 July 1954. The trap was placed with the orifice 2 m. above ground-level,

* Now at Department of Botany, Andhra University, Waltair, India.

about 250 m. from the sea, separated by a shingle bank from the western arm of Chichester Harbour (National Grid Ref. SU 755022). The trap was in a small plot of potatoes with a few small trees to the north-west and south-east. Extensive mud flats were exposed at low tide (Fig. 1).

The methods used for preparing slides for exposure and mounting for study were as described by Hirst (1953). After mounting unstained, the slides were scanned under the oil-immersion objective at distances along the trace (i.e. at right angles to the direction of the slide movement) corresponding to 2-hourly intervals. The number counted on a traverse $40\ \mu$ wide (determined with a graticule in the eye-piece) was then converted into an estimated number of spores per m^3 of air.

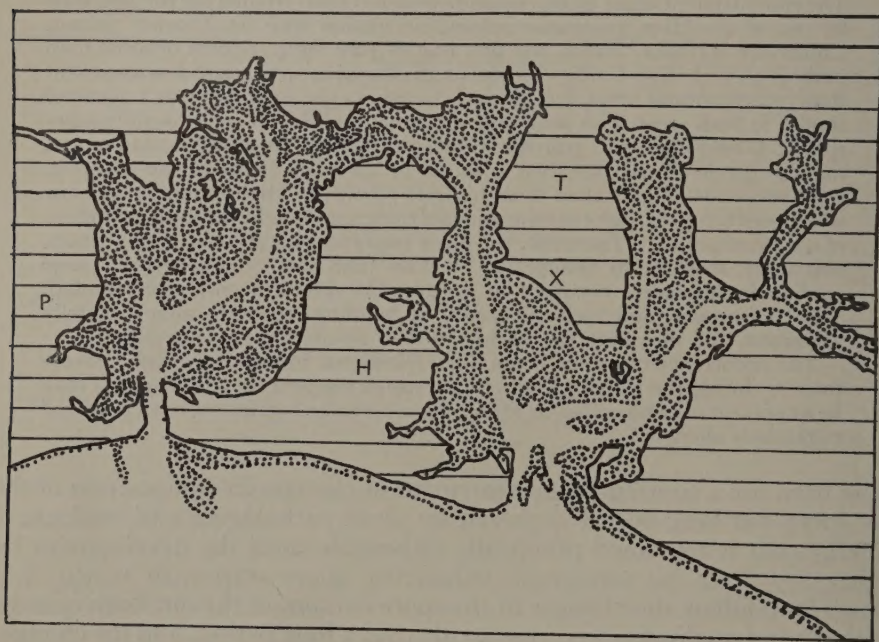


Fig. 1. Map of part of Chichester Harbour showing position of spore trap (X) in relation to mud exposed at low tide (dotted area). T, Thorney Island; H, Hayling Island; P, Portsmouth. Scale: $\frac{1}{2}$ in. to 1 mile. Based upon the Ordnance Survey Map with the sanction of the Controller of H.M. Stationery Office.

Air was sampled at the rate of 10 l./min. The slides were changed daily at about 10.00 hr. and the counts were made at 2-hourly intervals starting from 10.30 hr. each day. All times given are Greenwich mean time. (Data from 21.00 hr. 5 July to 09.30 hr. 6 July are missing because of a fault in operation.)

RESULTS

The automatic volumetric spore trap retains small spores with a high efficiency and therefore, as already pointed out by Hirst (1953), it reveals the occurrence of small and hyaline spore types, especially basidiospores, which have escaped the attention of most of the early workers. Gregory

(1952) and Gregory & Hirst (1952), working with the Cascade Impactor (May, 1945) and the Hirst trap, for the first time recorded the presence of basidiospores as a significant element of the air spora, especially during the early hours of summer and autumn mornings at Rothamsted Experimental Station. The basidiospore components of the air spora were studied in considerable detail by Hirst (1953), who grouped them into two categories: hyaline and coloured basidiospores.

We have attempted to study the individual components of these heterogeneous basidiospore groups by taking separate counts for *Sporobolomyces*, *Tilletiopsis*, 'hyaline', 'Red-brown' and 'Yellow' basidiospores. In addition, some other highly characteristic spore types such as *Ustilago*, *Cladosporium* and *Alternaria* (including *Stemphylium*) were counted separately. Still other spores were grouped as 'pollens', 'Powdery mildews', 'septate fusiform spores', and 'long filiform' spores. Fungus spores not included in any of the above-named groups, were counted under one group 'Others'. Colonies of the blue-green alga, *Gloeocapsa*, were also encountered on many slides, and these colonies and the number of cells present in each colony were also counted.

The changes in concentration of these spore types observed during the experimental period are shown in Figs. 2 and 3. The upper part of each figure shows sunshine, rainfall, wind velocity at 2 m. above ground-level, with temperature and relative humidity (R.H.), determined by a mercury-in-steel wet- and dry-bulb thermograph in a Stevenson screen 4 ft. above ground-level, plotted at hourly intervals. Changes in spore concentration per m.³ are plotted in the lower panels for comparison. Fig. 4 shows the mean diurnal periodicity of the individual spore types, plotted according to the method of Hirst (1953).

Sporobolomyces

Spores of *Sporobolomyces* were most abundant at night. They began to appear at about 22.30 hr. and increased rapidly to a maximum soon after midnight, then they decreased gradually till about 06.30 hr. and disappeared completely by about 09.00 hr. During their peak hours they occurred in high concentrations averaging about 10,000/m.³.

On dry days with moderate temperature and R.H. as on 5 and 12 July, the normal diurnal periodicity described above was maintained. When both wind speed and temperature were high but R.H. low, as on 3 and 4 July, not more than 400/m.³ were observed between 02.30 and 04.30 hr. Violent fluctuations of weather on the night of 1 July can be correlated with changes in spore concentration. Spores of *Sporobolomyces* appeared very early (18.30 hr.) and a concentration of about 15,000/m.³ was attained even by 20.30 hr. By 22.30 hr. their concentration increased to about 20,000/m.³, but by 00.30 hr. was reduced to 10,000/m.³. The early start on this day can be attributed to the very high humidity, and low temperature throughout the previous day with 2.8 mm. rain between 14.00 and 18.00 hr. The rapid reduction in spore concentration at midnight may have been due to the change in the wind speed, which was 5 m./sec. at 20.30 hr., increased to 8 m./sec. by 22.30 hr. and to 10 m./sec.

by 00.30 hr. when the concentration was nearly halved. A gradual decrease in concentration with increase in wind speed was also observed on the morning of 7 July.

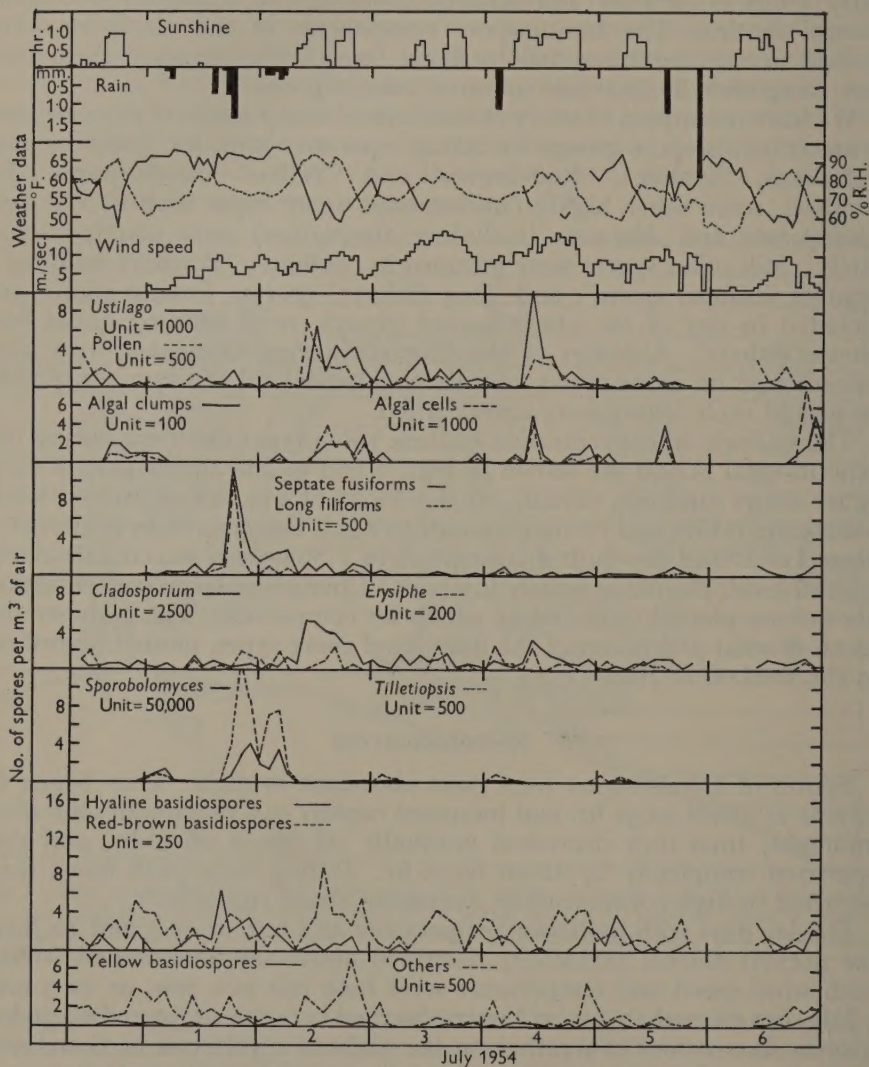


Fig. 2. Diagram showing changes in principal components of air spora of an estuary (Thorney Island) at 2-hourly intervals from 30 June to 6 July, 1954; with relevant meteorological data.

Highest concentrations of *Sporobolomyces* during the sampling period were observed on 9 and 10 July. Almost the whole of 8 July was cloudy with very high R.H. About 5.8 mm. of rain fell over a period of 9 hr. with a R.H. of 99 % continuously for 8 hr. on the morning of 9 July. On the evening of this day rain started again at about 19.00 hr. and drizzle continued till 03.00 hr. Wind speed was low in the morning, but by mid-

night it was high (about 11–14 m./sec.). Under these weather conditions *Sporobolomyces* was in the air in enormous numbers (about a million/m.³). These spores did not disappear during the day, and even at noon the

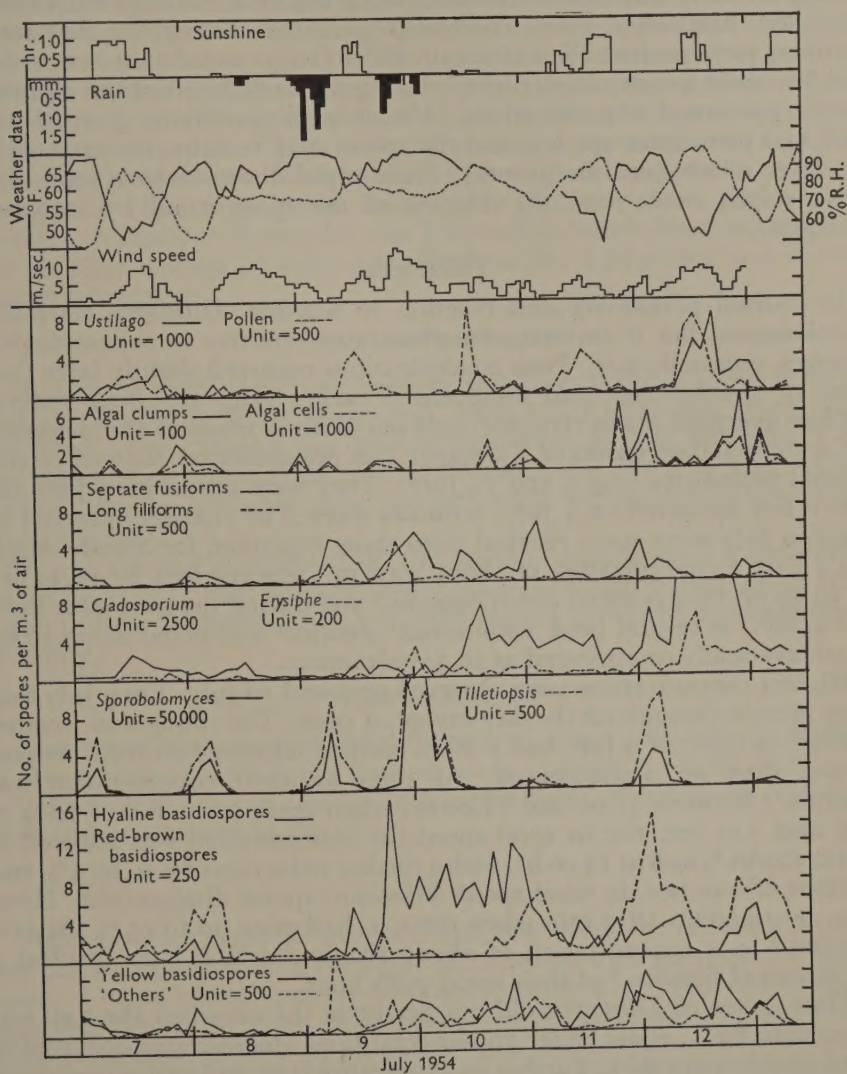


Fig. 3. As Fig. 2, but for 7–13 July 1954.

concentration was well above 1500/m.³. The slight fall in their concentration between 14.30 and 16.30 hr. might have been due to sunshine which increased the temperature and reduced the R.H. Another spell of rain then brought back optimum conditions. These changes of weather are clearly reflected in the catches. By 22.30 hr. the concentration reached about 54,000/m.³ and increased to 65,000/m.³ by midnight. Then a rain-fall of about 0.7 mm. with high wind speed reduced their numbers to

15,400/m.³. Within 4 hr., even with the relative humidity consistently at 99 % the concentration was reduced from 65,000 to 3700/m.³.

During the period studied spores of *Sporobolomyces* occurred abundantly during the early hours of the morning when high R.H. and low wind speed prevailed, and when these conditions persisted during the day large numbers were present even at noon. Hirst (1953) noted that heavy dew and low wind speeds which prevail during the early hours of the morning favour maximum concentrations. Under such conditions thermal and frictional turbulence are low and the spores may remain concentrated in the layer of air near the ground. Their rapid disappearance soon after dawn might result from the dilution of the spore cloud by increased turbulence.

Tilletiopsis

In diurnal periodicity and reaction to weather *Tilletiopsis* resembles *Sporobolomyces*, but it appears somewhat more sensitive to meteorological changes, especially R.H. Peak concentrations occurred slightly later than those of *Sporobolomyces* and disappeared earlier (Fig. 4). Peak concentrations averaged about 1600/m.³, only one-sixth as many as *Sporobolomyces*.

On normal days spores of *Tilletiopsis*, like *Sporobolomyces*, showed typical diurnal periodicity (e.g. 5 and 12 July). They were absent on 3 July and only a few appeared on 4 July, both dry days. The changes observed on 1 and 9 July were more marked than those described for *Sporobolomyces*. On 1 July a concentration of about 2000/m.³ was reached by 18.30 hr.; by 20.30 hr. they reached about 6500/m.³ and during the next 2 hr. there was a fall. A second peak with about 3800/m.³ was noted at 04.30 hr. which gradually disappeared as in *Sporobolomyces*.

Highest concentrations were observed on 9 and 10 July. On 9 July they were present throughout the day except at noon. The changes in weather during the night of 9 July had a more marked influence on their concentration than on *Sporobolomyces*. At 10.30 hr. their concentration was 1100/m.³; between 11.00 and 12.00 hr. when there was a fall of 10 % in R.H. and 2 m./sec. rise in wind speed the concentration was reduced to about 100/m.³; and at 13.00 hr. with a further reduction of R.H. by 5 % and increase of 2 m./sec. in wind speed *Tilletiopsis* spores disappeared. However, by 18.00 hr. after rain when the R.H. had gone up to 95 % they reappeared in a concentration of about 1600/m.³ which later reached a maximum of 6000/m.³ at their usual peak hour.

Thus spores of *Tilletiopsis* did not persist in the air when the R.H. was lower than 85 %, while under similar conditions *Sporobolomyces* occurred in considerable quantities. Further its appearance later and its disappearance earlier in the day indicates that *Tilletiopsis* requires much more humid conditions for sporulation than *Sporobolomyces*.

Large hyaline basidiospores

These were present in their lowest numbers at about noon and were at their maximum during the night time. Peak concentrations averaging about 850/m.³ occurred at 22.30 hr., much earlier than spores of *Sporobolomyces* and *Tilletiopsis*.

Their presence in the air seemed more dependent on weather, especially on rain and R.H., than the foregoing types. They persisted in relatively high concentrations on 9 and 10 July. Continuous heavy rain of about 8 mm. during the morning of 9 July almost washed these spores from the atmosphere, but again with the first showers that occurred in the evening concentrations of about $4400/m.^3$ were again established and they persisted through another cloudy day with some rain on 10 July until the normal periodicity was once again established on 11 July which was dry. Similarly, they occurred in high concentrations on the afternoon of 1 July immediately after rain. This behaviour favours the suggestion made by Gregory & Hirst (1952) that these spores probably come mainly from leathery, resupinate Agaricales and Tremellales which liberate them into the air after wetting by rain. On dry days like 3 July they were almost completely absent.

Red-brown basidiospores

Highest concentrations of these types usually occurred at about 02.30 hr. and the lowest between 08.30 and 14.30 hr. Unlike the spore types described above, red-brown basidiospores were present throughout the day though their concentration in the afternoon was low. The peak concentration, at night, averaged about $900/m.^3$, while the midday minimum was about $250/m.^3$.

During the period of this study the highest concentration was observed on the night of 11 July, and considerable numbers were caught on 30 June, 7 and 10 July. Large numbers were also present in the air on the afternoon of 2 July.

Yellow basidiospores

Peak concentrations at midnight averaged about $900/m.^3$, and minima of about $300/m.^3$ were found from 08.30 to 14.30 hr. on most days.

Yellow basidiospores, like the red-brown types, occurred on almost all days and at all times in the day.

Pollens

Pollens, which were all grouped into one category, occurred in very low concentrations during the night and reached their maximum at about noon. Lowest concentrations (average $30/m.^3$) were observed at about 04.30 hr. From 08.30 hr. their concentration increased gradually to a peak averaging $1500/m.^3$ by 12.30 hr. Their concentration then decreased till 18.30 hr. after which small quantities remained till next morning.

Concentration appeared to be greatly influenced by sunshine and temperature, especially during the peak hours. In general higher concentrations occurred on fine sunny days as on 11 and 12 July, both of which followed three nearly sunless days with intermittent rain, but on days with little or no bright sunshine their concentration remained low as on 1 and 8 July. As suggested by Hyde & Williams (1945) high concentrations might have been due to a holding back of anthesis on dull days and its release on the day after. Rain affects pollen concentration in the air both

indirectly in preventing or delaying the dehiscence of anthers by decreasing temperature and increasing the humidity, and directly by washing down pollen which is already in the air. The lowest concentration observed (on 8 July) can be attributed to the 0.4 mm. of rain which fell during the peak period between 12.00 and 14.00 hr. As pollen is usually liberated in day time rain during the day affects the concentration more than rain at night. For example, on 2 July even though there was 1 mm. of rain for nearly 4 hr. from 01.00 hr. normal pollen concentrations appeared at the usual time.

Cladosporium

Cladosporium spores were abundant on many days, with peak concentrations at about 10.30 hr. averaging 12,000/m.³ and reaching nearly 100,000/m.³ on some days. They occurred in low numbers at night and reached their minimum at about 04.30 hr. with an average concentration of about 2300/m.³.

Hyde & Williams (1953) considered that a temperature of about 60–65° F. is critical in determining the concentration of *Cladosporium* spores in the air. Within this range any rise in temperature increases the concentration and any fall decreases it markedly. During our study at Thorney Island maximum concentrations occurred on 12 July between 08.00 and 18.00 hr. when the temperature was within this range. The temperature was also within this range on the night of 11 July when the concentration of *Cladosporium* also was unusually high.

Transient increases in *Cladosporium* in the air spora when rain starts to fall have been noted by Ainsworth (1952) and Hirst (1953). Gregory (1954) using the portable spore trap studied the effect of showers of rain separated by dry intervals. At Thorney Island rain at peak hours had a large effect. Thus on 4 July when only 0.2 mm. of rain fell between 10.00 and 11.00 hr. a marked increase in *Cladosporium* was observed, whereas on 9 July with heavy rain both in the morning and in the evening and with bright sunshine from 09.00 to 14.00 hr. the concentration was normal. Similarly, on 5 July normal concentrations persisted through heavy rain after the peak hours. However, the number of occasions with rain is too small to justify any conclusions.

Long filiform spores

Filiform spores were present only immediately after rain. The highest concentration, 5200/m.³, was observed at 18.30 hr. on 1 July, immediately after the fall of 1.4 mm. rain in 1 hr. They were absent on dry days. Rain seems to be the major factor influencing the concentration of these spores, as they become more abundant with the continuation of rain, while after rain they soon disappeared even if the day was dull with low temperature and high R.H. These observations support the suggestion made by Hirst (1953) that the filiform spores are mostly the ascospores ejected into the air from mature asci after wetting by rain.

Septate fusiform spores

Spores of this type occurred in measurable numbers all through the period and showed a clear diurnal periodicity; their peak concentration, averaging about 850/m.³, occurred during the night.

Their liberation into the air was favoured by rain. The highest concentration observed (5700/m.³) occurred on 1 July at 18.30 hr. immediately after a heavy shower; with more light showers of rain during the night lower concentrations persisted till the morning of 3 July when conditions became dry. More rain occurred during 9 and 10 July and septate fusiform spores again attained high numbers.

This group differs in behaviour from long filiform spores, as even on very dry days they are not completely absent and show their normal periodicity.

Ustilago

Smut spores were found in very low concentrations in the early hours of the morning; they reached their peak by 10.30 hr. (averaging 1600/m.³) and remained in relatively high numbers till late in the afternoon. They then showed a gradual decrease reaching a minimum (average 200/m.³) by about 04.30 hr.

Comparatively high concentrations were observed on 2, 4 and 12 July at times when the wind velocity was high. Their complete absence on 9 July may have been an effect of rain.

Erysiphe

A few conidia of powdery mildews, probably belonging to the genus *Erysiphe* were encountered on these slides. Maximum numbers observed were on 12 July. Their diurnal periodicity was in general similar to that of *Ustilago*.

Alternaria and Stemphylium

These spore types were not at all common except on the 12 July when comparatively high concentrations were noticed; on all other days only a few were caught. Their periodicity resembled *Cladosporium* with a maximum at about noon.

'Others'

All fungus spores found on the slides other than those described above were counted under this group. Further identification of many of the constituents of this group is not possible. Many of them may have belonged to splash dispersed fungi whose spores occur in air during rain. Maximum numbers were found on 1, 2 and 9 July following showers of rain.

Colonies of Gloeocapsa

During the whole trapping period a blue-green alga identifiable microscopically as belonging to the Chroococcaceae (probably belonging to the genus *Gloeocapsa*) was found in the air (Gregory, Hamilton & Sreeramulu, 1955).

Over the period of 13 days an average of 110 colonies/m.³ of air was recorded with an average of 8 cells per colony. Diurnal periodicity shows maximum numbers at 22.30 hr. with an average of 210 colonies/m.³ and a minimum between 04.30 and 12.30 hr. with an average of about 30 colonies/m.³.

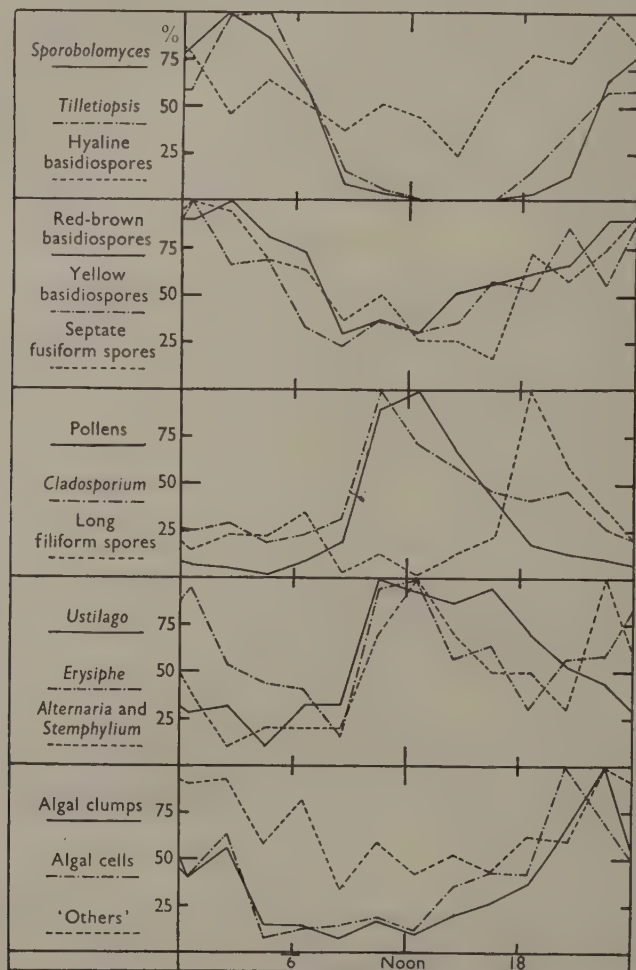


Fig. 4. Mean diurnal periodicity of components of air spora at Thorney Island, expressed as percentage of the peak arithmetic mean concentration.

Highest concentrations were observed on 12 July, while very low concentrations were found on 3 July. When sunshine, R.H. and temperature were about normal, as on 7 July, the concentrations were moderate. Rain seems to increase the numbers in the air, as on 2 and 6 July. Continued heavy rain which fell over a long period, especially that of 9 and 10 July

at first washed out all those present in the atmosphere, but continued humid conditions probably contributed to the extremely high concentrations observed on 11 and 12 July.

DISCUSSION

Changes in the categories of spores caught at different times of day in the estuary showed a general agreement with the findings for agricultural areas. Spores of some crop pathogens including *Ustilago*, *Alternaria* and powdery mildews, which occur in enormous numbers in agricultural areas, were present in very low numbers. Basidiospores, spores of *Cladosporium* and pollens were the dominant constituents of the air spora in this estuary. Concentrations of hyaline basidiospores, with their maxima in the early hours of the morning, were very much higher than those hitherto recorded elsewhere. The persistence of very humid conditions for a long period in this area is considered to favour higher numbers. Within a few hours of the start of rain the typical dry air spora was replaced by hyaline spores which included splash dispersed types, ascospores and basidiospores.

The work of Gregory (1954) and Last (1955 *a, b*) showed that *Sporobolomyces* and *Tilletiopsis* spores from the members of *Sporobolomycetaceae* present locally on the leaves of plants are among the principal components of transient clouds of hyaline basidiospores. Of the three major constituents of the hyaline basidiospore group of Hirst (1953), *Sporobolomyces* and *Tilletiopsis* occur regularly (the former in very high concentrations and the latter in low concentrations) with their peaks during the early hours of the morning, depending less on the weather conditions, while the third group—large hyaline basidiospores—occur in very low concentrations on dry days, but on days with rain and high relative humidity, they occur in large numbers. Coloured basidiospores derived probably from agarics and polypores were also commonest at night, but their occurrence depended less on weather than did hyaline basidiospores and their diurnal periodicities were also less pronounced. Much published work suggests that the fruit-bodies of many of these fungi with coloured spores, liberate spores at a relatively uniform rate, and that changes in temperature, water supply and light may not greatly influence the rate of spore liberation. Our data fail to throw more light on this problem, and much further work is needed on the influence of weather conditions on spore liberation in the larger basidiomycetes. Our data show clearly that the individual components of these two basidiospore groups (hyaline and coloured) differ from each other in the amplitude of their diurnal periodicity, and agree well with Hirst's (1953) data for Rothamsted.

The occurrence of unicellular algae in the air or in air-borne dust has been recorded by several investigators, but so far no detailed study on the algal components in the air has been made. Although the concentrations of *Gloeocapsa* recorded from this area are small in comparison with those normal for pollen or fungus spores, their occurrence in considerable numbers at least for some weeks in air in certain localities, warrants their further study as potential allergens, especially as their cells have thick sheaths of mucilage (Gregory *et al.* 1955).

So far as our data go the air spora in the estuarine area studied differs only quantitatively from that of an inland agricultural area.

Acknowledgement is made to the Director-General of the Meteorological Office for the assistance given by the meteorological staff of Royal Air Force, Thorney Island.

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(Accepted for publication 9 April 1957)

Trans. Brit. mycol. Soc. **41** (2), 157-164 (1958).

INOCULUM POTENTIAL AS A FACTOR LIMITING LETHAL ACTION BY *TRICHODERMA VIRIDE* FR. ON *ARMILLARIA MELLEA* (FR.) QUÉL.

By S. D. GARRETT

Botany School, University of Cambridge

As originally reported by D. E. Bliss, fumigation with carbon disulphide of soil containing roots infected by *Armillaria mellea* results in a notable increase of *Trichoderma viride*, which has a tolerance of carbon disulphide higher than that of most other soil fungi; the augmented population of *T. viride* invades the fumigated roots, but there is still an open question as to what proportion of the *A. mellea* mycelium is killed by direct fungicidal action of carbon disulphide and what proportion is killed by lethal action of *T. viride* following fumigation. The latter fungus is not the only one thus selected by fumigation; *Penicillium wortmanni* is still more resistant to carbon disulphide, and developed prominently on the surface of fumigated wood segments in a previous investigation.

The experiment described was designed to determine whether *T. viride* acting on its own could kill *A. mellea* in woody inocula, and to what extent the lethal action of *T. viride* was governed by its inoculum potential in the soil. Incubation of inoculum segments for 23 days in a pure culture of *T. viride*, grown on autoclave-sterilized soil, resulted in a 95% kill of *A. mellea*; dilution of the pure culture to $\frac{1}{4}$ and $\frac{1}{8}$ strengths with unsterilized soil caused a progressive reduction in the lethal effect of *T. viride* upon *A. mellea*. The greatest lethal activity of *T. viride* against *A. mellea* previously observed in a fumigated and then incubated soil did not exceed that produced by dilution of the pure culture of *T. viride* to $\frac{1}{4}$ strength in this experiment. Incubation of inocula of *A. mellea* in a pure culture of *P. wortmanni* for the same period resulted in no loss of viability, but rather in a slight increase in rate of rhizomorph growth from the inocula thus treated.

The behaviour of *Trichoderma viride* Pers. ex Fr. as a parasite of other soil fungi has attracted much attention since its original discovery and elucidation by Weindling (1932, 1934). The practical exploitation of these potentialities for the control of root disease fungi has proved a more difficult and intricate task than had originally been supposed, but at least it can be said that we have now a clearer view of the obstacles ahead. As Brian (1957) has implied in his masterly survey of the ecological significance of antibiotic production in the soil—a subject to which he and his associates have contributed so much of our detailed knowledge—the problem of biological control of soil-borne diseases is essentially a problem in applied microbial ecology.

The immediate concern of this paper is with a promising new approach to this general problem by Bliss (1951), who observed that when sections of citrus roots infected by *Armillaria mellea* had been fumigated in soil with carbon disulphide, an extensive invasion of the fumigated roots by *T. viride* then followed. Bliss attributed this striking increase in the population of *T. viride* to the selective effect of carbon disulphide, which

was thought to have eliminated other fungi and other soil micro-organisms that were less tolerant of the fumigant than was *T. viride*. On the basis of this and other types of observation and experiment, Bliss concluded that the killing of *A. mellea* in infected roots by soil fumigation was due not to direct fungicidal action by carbon disulphide, but to an indirect, microbiological effect of fumigation, i.e. to the increase of *T. viride* in the fumigated soil.

In a series of experiments designed to test Bliss's hypothesis, I obtained conclusive evidence for a microbiological after-effect of soil fumigation with carbon disulphide, as postulated by Bliss (Garrett, 1957). Small woody inocula of *A. mellea* were incubated for 3 weeks in a soil that had first been fumigated with carbon disulphide at the rate of 828 p.p.m. and was then kept for 3 weeks before use, to allow the fumigant to disperse. Loss of viability by *A. mellea* as a result of incubation in the fumigant-free soil did not exceed 30 %, however, whereas direct fumigation of *A. mellea* inocula in soil with one-quarter this dose (207 p.p.m.) was sufficient for 100 % kill of the fungus by direct fungicidal action. I therefore concluded that, when infected roots are fumigated in soil, some killing of *A. mellea* by direct fungicidal action must occur but that, in massive roots too large for complete penetration by the fumigant, *T. viride* and/or other fungi may finish what direct fungicidal action by carbon disulphide has begun.

The purpose of the present communication is first to confirm one of Bliss's most important observations, and, secondly, to provide evidence for an assumption that I had to make in order to reconcile this observation with others. Bliss found that if root sections infected by *A. mellea* were buried and incubated in a pure culture of *T. viride* grown on autoclave-sterilized soil, then *A. mellea* was completely killed. I was unable to agree with Bliss's acceptance of this observation at its face value as evidence for his hypothesis, for the following reason. When *T. viride*, or any other fungus, is grown in pure culture on completely sterilized soil, the whole reserves of the substrate are at its disposal for growth, and for production of growth products such as antibiotics. In a soil that is admittedly only partially sterilized by fumigation with carbon disulphide, *T. viride* may be apparently the 'dominant' fungus, but nevertheless it is sharing the substrate with the other, but less noticeable, fumigant-resistant fungi, bacteria and assorted microbes. I therefore concluded: 'The inoculum potential of a dominant fungus in a natural or semi-natural community of soil micro-organisms is therefore certain to be lower, and probably very much lower, than that of the same fungus grown in pure culture on completely sterilized soil.' I have defined this term 'inoculum potential' as 'the energy of growth of a parasite available for infection of a host at the surface of the host organ to be infected' (Garrett, 1956a, p. 196, and see index for other references). Substantial evidence has been reported by a number of workers in support of the view that a certain level of inoculum potential in a root-infecting fungus is essential for successful invasion of a host root, in the course of which passive host resistance has to be negotiated and active host resistance tolerated or neutralized. It seems reasonable to suppose that an adequate inoculum potential may be equally essential for

the successful parasitism of one fungus by another fungus, in this instance of *A. mellea* by *T. viride*, and evidence for this view is reported below.

This summary of the situation, *T. viride* versus *A. mellea*, requires amplifying in one further respect. It will be apparent that I have followed Bliss in the assumption that, of the soil fungi selected for development in the fumigated soil by their tolerance of carbon disulphide, *T. viride* is the most likely to be responsible, at least for the major part of the indirect, microbiological effect of fumigation upon *A. mellea*. This assumption is founded not only on the more general studies of parasitic activity by *T. viride*, but also on Aytoun's (1953) particular investigation of the parasitism of *A. mellea* by *T. viride* in dual culture. Ten isolates of *T. viride* were opposed in turn to each of two isolates of *A. mellea* on parallel smears of malt agar, adjusted to pH 3.4, 5.1 and 7.0, respectively, in van Tieghem cells. All ten isolates of *T. viride* behaved similarly, as follows. On malt agar at pH 3.4, the hyphae of *T. viride* coiled around those of *A. mellea*; occasionally actual penetration of the hyphae of *A. mellea* by those of *T. viride* was observed. *T. viride* was stimulated by this contact to branch and to produce conidia. The hyphae of *A. mellea* eventually deteriorated visibly, and the colony could not be subcultured. When the two mycelia met on the glass surface, away from the agar, parasitism of the *A. mellea* hyphae occurred only at points of actual contact with the hyphae of *T. viride*. On agar at pH 5.1, these effects were less pronounced, and on agar at pH 7.0 there was no obvious effect of *T. viride* upon *A. mellea*.

These observations by Aytoun on the differential effect of the pH value of the agar substrate confirm again the original observation by Weindling (1934) that parasitic action by *T. viride* is most active in an acid medium; this has been related to observations by Weindling and by later workers that the stability of both gliotoxin and viridin is greatest at a low pH value. Brian (1951) has concluded that most strains of *T. viride* probably produce a mixture of these two antibiotics, the proportion varying from strain to strain. More recent work (Dr P. W. Brian, personal communication) has shown that most isolates of this fungus obtained at random from the soil produce amounts of antibiotic that, if detectable, are very small by comparison with that produced by strains specially selected for production either of gliotoxin or of viridin in the laboratory. It is all the more remarkable, therefore, that the ten isolates of *T. viride* obtained by Aytoun, presumably by random isolation from the soil, should all have been able to parasitize hyphae of *A. mellea*, under suitable conditions (i.e. a substrate of pH 3.4). This suggests that gross production of one or other antibiotic by a strain of *T. viride* is not essential for its parasitic activity, though production of a certain minimal concentration, not easily detectable by present methods, may quite possibly be so. Without this second supposition, indeed, it is difficult to understand why 'non-antibiotic-producing' strains, as well as gross producers, show a marked optimum for lethal action at low pH values.

Notwithstanding this accumulation of circumstantial evidence for the view that *T. viride* is the agent responsible for the post-fumigation effects of treated soil upon *A. mellea*, it is necessary to state that, in my previous

investigation, one other fungus was favoured at least as much as was *T. viride* by soil fumigation, and was prominent on the surfaces of inoculum segments and of uninoculated wood 'baits' fumigated in the soil. This was a conspicuous yellow mould, *Penicillium wortmanni* of the *P. luteum* series (Raper & Thom, 1949); in an earlier investigation of the same soil, Evans (1955) found that, at higher dosages of carbon disulphide, *P. luteum* and *Aspergillus fischerii* replaced *T. viride* as the dominant fungi developing on Warcup soil plates made from the fumigated soil. When Evans tested these ascospore ascomycetes in pure culture for tolerance to carbon disulphide, he found them to be markedly more resistant than was *T. viride*, which in turn was more resistant than the other soil fungi tested. It therefore seemed advisable to include *P. wortmanni* in the experiment to be described below, in which woody inocula of *A. mellea* were incubated for 3 weeks in pure cultures of *T. viride* and of *P. wortmanni*, respectively, and in one or more dilutions of each pure culture with unsterilized soil. An additional reason for including *P. wortmanni* in this trial arises from the announcement by Brian, Curtis, Hemming & Norris (1957) that they have isolated a new antibiotic, which they name wortmannin, from an isolate of *P. wortmanni* from Malaya. Wortmannin was found to be remarkably specific in its antifungal properties; of thirty-eight fungal species (which did not include *A. mellea*) tested for sensitivity by spore germination tests, twenty-one were unaffected by 100 µg./ml. wortmannin, whereas five species were inhibited by concentrations over the range 0.4–3.2 µg./ml.

EXPERIMENTAL WORK

Full details have already been given of methods for preparation of small, woody inocula of *A. mellea* in pure culture, and for testing the viability and vigour of the mycelium therein, after subjection to various experimental treatments, by measurements of weekly growth increments of rhizomorphs through tubes of moist soil (Garrett, 1956*b*, 1957). For this experiment, inocula were prepared from shoots of pollarded *Salix fragilis* not exceeding 1.75 cm. diam., which were sawn into segments 2.5 cm. long (mean fresh weight per segment was 4 g.). Inoculum segments were incubated in the culture flasks after inoculation with *A. mellea* for 2½ months at 25° C., and thereafter for nearly 1½ months further at laboratory temperature (20–23° C.) before use.

The soil employed for incubation treatments of the *A. mellea* inoculum segments was the same as that employed previously for this investigation—Kettering loam (pH 6.1 for this sample). For the preparation of pure cultures of *T. viride* and *P. wortmanni*, respectively, Kettering loam at a moisture content of 50 % m.h.c. was sterilized in 170 g. lots in conical flasks (500 ml.) for 1 hr. at 27 lb. (1.84 atm.) pressure; after inoculation with the respective fungi, flasks were incubated at 25° C. for 19 days. The culture of *T. viride* had been isolated a few weeks previously by my colleague Dr J. Rishbeth, from a mass ascospore plating of the perfect stage (*Hypocrea rufa*, see Bisby, 1939) that had developed on a pine stump treated with formalin about a year earlier. The culture of *P. wortmanni*, which had been identified by my colleague Dr N. F. Robertson, and

confirmed by Prof. K. B. Raper, was isolated in May 1956, from wood baits fumigated in soil with carbon disulphide.

After this period of incubation, some aliquots of the pure cultures of *T. viride* and *P. wortmanni*, respectively, were diluted by thorough mixing with unsterilized Kettering loam at a moisture content of 40 % m.h.c., according to the following scheme:

Series C. Unsterilized Kettering loam: control.

Series T. Pure culture of *T. viride* on autoclaved Kettering loam.

Series $\frac{1}{4}$ T. One vol. pure culture of *T. viride*: 3 vol. unsterilized soil.

Series $\frac{1}{8}$ T. One vol. pure culture of *T. viride*: 7 vol. unsterilized soil.

Series P. Pure culture of *P. wortmanni* on autoclaved Kettering loam.

Series $\frac{1}{4}$ P. One vol. pure culture of *P. wortmanni*: 3 vol. unsterilized soil.

In each of these six experimental soils (400 g. lots of each) was buried a batch of twenty inoculum segments of *A. mellea*; each series was incubated in a 2 lb. glass jam jar at laboratory temperature (21–24° C.) for 23 days. At the end of this incubation period, inoculum segments were removed from the jars, brushed clean, and set-up in soil growth tubes for a test of their capacity for rhizomorph production and growth. The method is fully described (in Garrett, 1956*b*, 1957), but it is necessary to note that the soil employed in the growth tubes was not Kettering loam, but a light loam from my own garden at Cambridge, which had been adopted as a standard filling, on account of its ease of handling and near-optimum characteristics, at a moisture content of 60 % m.h.c., for rhizomorph production and growth. Rhizomorph growth tubes were incubated at 25° C.; the first measurements of the extent of rhizomorph growth from either end of each inoculum segment, as seen through the glass wall of the tube, were made 13 days after setting-up. Thereafter, weekly measurements of the extent of rhizomorph growth were made, final observations being recorded after 76 days.

The information from the rhizomorph growth test is presented below. Table 1 shows the number of inoculum segment-ends producing rhizomorphs over the 76-day observation period, out of a total of forty possible. Table 2 shows the mean weekly growth increments of rhizomorphs calculated for all the segment-ends that did produce rhizomorphs (i.e. those that did not are excluded from calculation of the means). As only a single inoculum segment produced rhizomorphs (32 mm. growth from one end and 22 mm. from the other, after 76 days) out of the twenty incubated in a pure culture of *T. viride*, this series is missing from Table 2. To assist in the interpretation of this table, it is necessary to add that weekly growth increments of rhizomorphs from small woody inocula buried in soil invariably decline progressively with time (Garrett, 1956*b*). The initial growth rate depends on the size of the inoculum segment. The subsequent decline has been attributed to (1) gradual consumption of nutrient reserves in the inoculum through fungal respiration and growth, (2) increasing competition for nutrients between the main growing apex of each rhizomorph and its subordinate branch apices, and possibly also to similar competition between early and later-produced rhizomorphs.

Considering first the results given in Table 1, it will be seen that only one inoculum segment of twenty produced rhizomorphs in series T, buried

in a pure culture of *T. viride*. In view of the fact that in the control series C, buried in unsterilized soil, 38/40 segment-ends had produced rhizomorphs after 13 days, and 40/40 after 20 days, it seems reasonable to presume death of *A. mellea* in 19/20 segments of series T that had failed to produce rhizomorphs from either end after 76 days. The 95 % kill thus obtained agrees closely enough with the 100 % kill reported by Bliss (1951) for a similar treatment. In series $\frac{1}{4}$ T, 10/40 inoculum segment-ends failed to produce rhizomorphs after 76 days, but only 2/40 similarly failed in series $\frac{1}{8}$ T. Burial of inoculum segments in a pure culture of *P. wortmanni*, on the other hand, produced no inactivation of *A. mellea*; on the contrary, rhizomorphs were put out by this series of inoculum segments rather earlier than from the control series buried in unsterilized soil.

Table 1. *Production of rhizomorphs by A. mellea from individual inoculum segment-ends*

	No. segment-ends producing rhizomorphs after:										No. segments failing to produce rhizomorphs after 76 days	
	Days from setting-up growth-tubes										(a) From either end	(b) From one end only
	13	20	27	34	41	48	55	62	69	76		
Series C, unsterilized soil, control	38	40	—	—	—	—	—	—	—	—	0	0
Series T, pure culture <i>T. viride</i>	1	1	1	1	1	1	1	1	2	2	19	0
Series $\frac{1}{4}$ T, $\frac{1}{4}$ <i>T. viride</i>	18	22	26	26	26	26	27	30	30	30	4	2
Series $\frac{1}{8}$ T, $\frac{1}{8}$ <i>T. viride</i>	30	36	37	37	37	37	37	37	37	38	1	0
Series P, pure culture <i>P. wortmanni</i>	40	—	—	—	—	—	—	—	—	—	0	0
Series $\frac{1}{4}$ P, $\frac{1}{4}$ <i>P. wortmanni</i>	36	39	40	—	—	—	—	—	—	—	0	0

Table 2. *Mean weekly growth increments of rhizomorphs (mm.)*

	Weekly growth periods					Mean (± 0.50)
	1	2	3	4	5	
Series C, unsterilized soil, control	24	14	13	9	7	13
Series $\frac{1}{4}$ T, $\frac{1}{4}$ <i>T. viride</i>	13	4	3	1	0	4
Series $\frac{1}{8}$ T, $\frac{1}{8}$ <i>T. viride</i>	17	7	4	4	3	7
Series P, pure culture <i>P. wortmanni</i>	28	19	15	10	4	15
Series $\frac{1}{4}$ P, $\frac{1}{4}$ <i>P. wortmanni</i>	20	8	11	10	8	11
Mean (± 0.50)	20	10	9	7	4	—

Standard error for individual means = 1.12.

From Table 2, we can obtain an estimate of the vigour of *A. mellea* in those inoculum segments, or half-segments, in which it had not been inactivated completely. This estimate can be found as a single figure for each series, in the form of the mean growth rate for the whole period of 5 weeks for which weekly growth increments are given in the table. An analysis of variance performed on these data shows that all differences between mean rhizomorph growth rates for the whole 5-week period are

significant; each mean is based on a total of 200 measurements. For $C - \frac{1}{4}T$, $P = 0.001$; for $C - \frac{1}{8}T$, $P = 0.01$, and for $\frac{1}{8}T - \frac{1}{4}T$, $P = 0.02$. Thus the incubation of inoculum segments in diluted cultures of *T. viride* has produced significant reductions in rhizomorph growth rate from the surviving inocula, and the magnitude of the reduction has varied inversely as the dilution of *T. viride*.

The burying of inoculum segments in a pure culture of *P. wortmanni* has shown conclusively that this fungus, which develops prominently on the surface of inoculum segments and wood baits fumigated in Kettering loam with a sufficient dosage of carbon disulphide, is most unlikely to be responsible for any part of the microbiological after-effect of soil fumigation on *A. mellea*. This after-effect can now be attributed with more confidence to *T. viride*. It is interesting to note that incubation of inoculum segments in a pure culture of *P. wortmanni* has in fact produced a slight but significant ($P = 0.05$) increase in rhizomorph growth rate over the 5-week period, as compared with that for inoculum segments of the control series C. This increase cannot be attributed with any certainty to growth-products of *P. wortmanni*, however; it may merely mean that *P. wortmanni* is even less harmful to the vigour of *A. mellea* in the inoculum segments than is the normal microflora of unsterilized soil, which at least may compete more vigorously than does *P. wortmanni* with the mycelium of *A. mellea* for available nutrients, if it does nothing more.

DISCUSSION

The foregoing demonstration that the lethal action of *T. viride* upon *A. mellea* in woody inocula depends upon the inoculum potential of *T. viride* in the surrounding soil enables us to interpret more precisely my earlier observations (Garrett, 1957). In one of these experiments, incubation of inoculum segments of *A. mellea* for 23 days in a soil previously fumigated with carbon disulphide resulted in a loss of rhizomorph-producing capacity by 12/40 inoculum segment-ends, with a slight but significant reduction in rhizomorph growth rate, by comparison with the controls, from those segment-ends that did produce rhizomorphs. This result compares fairly closely with that obtained in the present experiment by burying inoculum segments for the same period in a mixture of 1 vol. pure culture of *T. viride* to 3 vol. unsterilized soil; 10/40 inoculum segment-ends were inactivated, but the reduction in rhizomorph growth rate (by comparison with the control series) from the remaining thirty segment-ends was much greater than that observed after incubation in the fumigated soil of the earlier experiment.

Although the experiment described in this paper has been carried out with only one isolate of *T. viride*, it is relevant to add that this isolate was chosen quite at random, being the only one recently brought into pure culture that happened to be available in the laboratory. The isolate had been obtained by Dr Rishbeth from a pine stump treated with formalin, and could therefore have been 'selected' for resistance to formalin, but it was certainly *not* selected on the basis of particular capacity for antibiotic production.

The essential and most valuable contribution made to this problem by Bliss has been his demonstration that the selective action of fungicidal fumigants offers a means of increasing artificially the soil population of *T. viride*—an increase that may eventually be exploited for the control of root disease fungi. The experiment that I have described in this paper has shown that the lethal parasitic activity of *T. viride* is related directly and quantitatively to its inoculum potential in the soil.

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(Accepted for publication 28 June 1957)

SUCCESSION OF FUNGI ON DECAYING STEMS OF *AGROPYRON REPENS*

BY H. J. HUDSON* AND J. WEBSTER

Botany Department, The University, Sheffield

(With 16 Text-figures)

The succession of fungi on decaying stems of *Agropyron repens* has been followed during a 19-month period after flowering. The patterns of fungal distribution follow broadly that already outlined for *Dactylis glomerata*. The colonization of flowering and non-flowering tillers in *Agropyron* is very similar. Isolations from decaying stems are briefly described.

Webster (1956*a*, 1957) has pointed out that little is known about the succession of fungi and other organisms which bring about the decay of plant material before it reaches the soil. He has described the pattern of colonization by fungi of flowering stems of cocksfoot grass (*Dactylis glomerata*) during a 2-year period following flowering. A number of distribution patterns for the more common fungi were distinguished:

(1) *Cladosporium herbarum*, *Alternaria tenuis*, *Epicoccum purpurascens*, *Leptosphaeria microscopica* and *Pleospora vagans* colonize leaves at all levels, but only persist in the fruiting condition on the uppermost three internodes until the end of the following summer.

(2) *Acrothecium* sp. fruits on basal internodes for the first few months after flowering.

(3) *Leptosphaeria nigrans* and *Mollisia palustris* fruit on the lower internodes in the summer following flowering. The fruiting of these fungi is restricted to the summer months.

(4) *Tetraploa aristata* and *Helminthosporium hyalospermum* also begin to fruit on basal internodes in the summer following flowering, but continue fruiting later and are particularly common on old collapsed stems.

(5) *Mycosphaerella recucita* and *Selenophoma donacis* fruit on the upper internodes of standing stems in the summer following flowering.

Evidence was obtained showing that the distribution of certain of these fungi was determined by the water content of the stems. For example, fungi in groups 2-4, normally confined to the base of standing stems, fruited on the upper internodes of stems laid on the ground. Measurements of the water content of standing stems showed consistently high values for lower internodes, and fluctuating values for upper internodes, whose water content was often below 15 %. It therefore seemed possible that the colonization pattern of fungi growing on cocksfoot stems might be determined primarily by the tussock habit of this grass; the crowded basal leaves of the tussock providing a habitat with a consistently higher

* Now at University of Oxford, Botany Department, South Parks Road, Oxford.

humidity than in the air surrounding the upper internodes. To test this idea it seemed desirable to follow the colonization of a grass of different habit, but of comparable stature, and *Agropyron repens* was chosen as a suitable grass which could be obtained from varied situations.

Although physical conditions such as differences in humidity may help to dictate the course of the succession, it seemed possible that differences in the fungal flora of upper and lower internodes might also be in part determined by nutritional differences. Since differences in the nutritional status of upper and lower leaves might be associated with physiological changes connected with flowering and seed-formation, it was felt to be of interest to compare the colonization of flowering stems in *Agropyron* with the colonization of sterile tillers, which often grow to the same height as flowering stems.

A further extension of the methods used by Webster is the use of a technique for isolating fungal mycelia from washed segments of tissue, which provided information about the distribution of fungi not necessarily in the fruiting condition.

MATERIALS AND METHODS

Localities

Agropyron stems were collected at monthly intervals from the following localities:

(a) *Quiet Lane, Fulwood, near Sheffield*, an area described by Webster (1956a). It is a small strip of rough grassland dominated in the area studied by *Agropyron*, but beyond this area *Deschampsia caespitosa* is the dominant grass. The ground is damp and frequently becomes waterlogged in winter.

(b) *University Botany Department Experimental Gardens, Tuptonville Road, Sheffield*, a small area dominated by *Agropyron* at the northern foot of a wall. Flowering tillers here collapsed earlier than in the other localities.

(c) *Lindrick Common, near Worksop, Notts*: the area studied lies in a disused Magnesian Limestone quarry to the north side of the A. 57 on the Worksop side of Lindrick Golf House. In contrast to Fulwood the area is well drained. Part of the area was burnt in May 1955.

Methods

(a) *Recording presence of fungi*. Similar techniques to those used by Webster (1956a) were adopted. Each monthly sample consisted of 10 stems. After incubation for 2 days in a damp chamber, the stems were scrutinized under a low-power binocular microscope and the distribution of fruit-bodies of fungi on each internode was recorded, following the convention shown in Fig. 1. Histograms were prepared indicating the frequency of the more common fungi in the sample at the particular internode level. It was found possible to collect samples representing a 19-month period from the time of flowering. By collecting 10 stems of the current season's growth and 10 stems from the previous season, the examination was condensed into 12 months.

(b) *Isolation technique.* Two stems were selected from some of the monthly samples from Lindrick and from the Gardens for isolation of fungi. 3-6 mm. pieces of leaf sheath and stem from each internode were washed separately in a modification of the apparatus described by Chesters (1948).

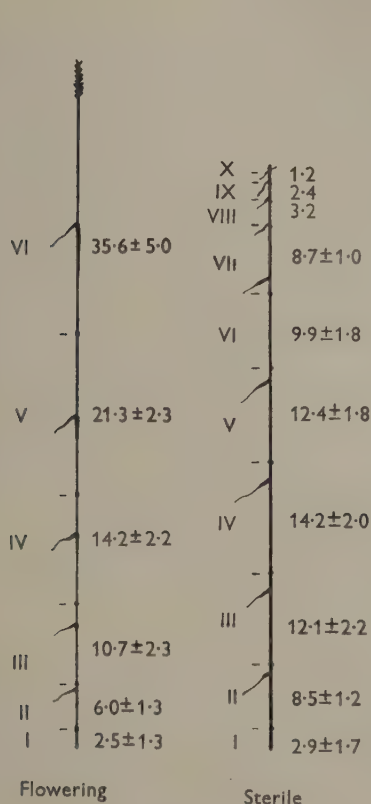


Fig. 1

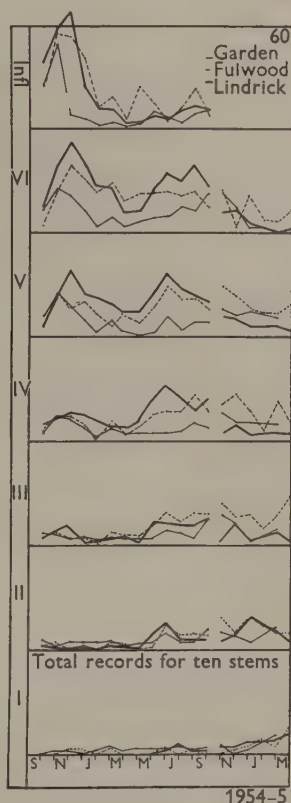


Fig. 2

Fig. 1. Comparison of internode length in flowering and sterile tillers of *Agropyron repens*. Roman numerals show the convention used in labelling. For purposes of comparison internodes I, II, III and IV of both series have been taken as equivalent. Internodes V and VI of sterile tillers have been taken as equivalent to V of flowering tillers, and all leaves above VII of sterile tillers have been taken as equivalent to VI of flowering tillers. Mean lengths (sample of 30 tillers) in cm. are also shown.

Fig. 2. Variation in records of fungi from a monthly sample of 10 stems. The vertical lines shown at internode I show the time of collapse of the majority of flowering tillers in each locality. After this time only collapsed tillers were collected.

In our technique seven washing units were arranged in parallel. The suspension of grass segments was agitated by bubbling air through sterile water which was changed at frequent intervals. The washed segments were suspended in a layer of maize-extract agar in Petri dishes. Bacterial contamination was reduced by the addition of Rose Bengal (concentration 35 mg./l.), 50,000 i.u. penicillin and 30,000 i.u. streptomycin per l. of medium were also added in aqueous solution before the agar was poured.

The plates were examined at intervals up to 5 weeks. After this time sterile mycelia were transferred to oatmeal agar slopes and examined at further intervals for evidence of sporulation.

THE HOST PLANT

Floral initiation in *Agropyron* occurs early in May as shown by Sharman (1947), and inflorescence tillers can be distinguished at this time by their stouter appearance and the presence of young inflorescences on dissection. Following the formation of inflorescence initials an acceleration of growth takes place, and the 3-6 unfolded leaves of the flowering stem exert as the internodes extend. Flowering begins in late July and extends into August. By this time all the leaves below internode IV (see Fig. 1) have turned brown and withered, whilst leaf IV is becoming senescent. Thus only the two uppermost leaves (V and VI) are functional at flowering, although the sheaths of leaves III and IV may still be green. Leaf IV unfolds about the end of May so that its functioning period is about 2 months. By early June, leaf V is mature and begins to wither early in July when leaf VI is fully functional, and this in turn ceases to function by mid-August. Once maturity is reached, senescence is more rapid in the upper leaves and leaf sheaths. The stems of internodes V and VI wither rapidly after senescence of the leaves, but the stems of internodes I-III may remain green until the following spring, and may give rise to new tillers. The delayed senescence of the lower stem segments may explain their apparent resistance to colonization by certain fungi.

Water content of stems

At intervals throughout the period of study, the water content of stem segments was determined, by weighing them in the field, and after drying, on a torsion balance. The data show a similar trend to the determination for *Dactylis* by Webster (1956*a*), but are somewhat complicated by the earlier collapse of *Agropyron* tillers. Typical results are shown in Table 1.

Table 1. *Water content (% dry weight) of Agropyron stems collected from Fulwood: mean values from three determinations*

Inter- node	1954			1955					
	Oct.	Nov.	Dec.	Jan.	Mar.	Apr.	May	June	Aug.
VI	107.3	17.8	44.8	132.6	22.6	16.4	9.7	46.4	86.4
V	106.6	32.3	87.5	166.8	65.8	16.4	8.9	110.1	79.9
IV	142.3	74.6	105.9	202.6	231.6	19.6	12.0	125.6	111.9
III	146.1	160.4	125.3	230.4	181.6	36.2	10.9	74.2	89.0
II	147.7	186.7	113.5	213.5	147.5	60.9	24.6	86.2	179.0
I	111.0	122.7	97.5	142.0	—	121.9	68.3	176.9	280.0

The data illustrate the consistently higher water content of the lower stem segments as compared with the upper. The water contents of the upper internodes may fall to values approaching 15 % which is probably close to the lowest limit at which fungal growth is possible (see Webster, 1956*a*).

SUCCESSION OF FUNGI ON DECAYING STEMS

In spite of differences between the growth habit and time of flowering in *Dactylis* and *Agropyron* the pattern of colonization is very similar. About 170 species of fungi were encountered in the three localities, many of them already known from *Dactylis*. Although most of the fungal groups recognized by Webster (1957) were present, *Acrothecium* sp. was not found. Other fungi were more common on *Agropyron*, for example *Pleospora trichostoma* which was only occasionally found on *Dactylis*, and *Ophiobolus herpotrichus* which was not recorded from *Dactylis*.

The successional pattern on *Agropyron* will be illustrated by a series of figures giving frequencies of selected species. The general outline is illustrated by Fig. 2, which shows the variation in number of records made in the three localities during the period 1954–55. The numerous records from the upper internodes during September to November reflect the high frequency of fungi in group 1 (see below), and a second peak the following summer at these levels is also largely due to the frequency of occurrence of group 1 fungi.

Group 1. *Cladosporium herbarum*, *Alternaria tenuis*, *Epicoccum purpurascens*, *Leptosphaeria microscopica*, *Pleospora vagans* and *Ramularia* sp. have similar distribution patterns and these are illustrated by frequency diagrams of *Cladosporium herbarum* (Figs. 3–5) and *Alternaria tenuis* (Figs. 6–8).

The most striking feature of the distribution of group 1 fungi on *Agropyron* is the similarity to their distribution on *Dactylis*. As in *Dactylis* all these fungi can be found fruiting on the lower leaves (especially on the blades) during the early summer months, but do not persist there. As the upper leaves become moribund they too become colonized by these fungi which persist, however, at this level until the following summer. Clearly there is some difference in the nature of upper and lower internodes of both *Dactylis* and *Agropyron* in spite of their different growth habit. The distribution of group 1 fungi on non-flowering tillers of *Agropyron* resembles the distribution on flowering stems (see Figs. 4, 5, 7 and 8, showing the distribution of *Cladosporium herbarum* and *Alternaria tenuis* on inflorescence and sterile tillers), and this suggests that physiological changes associated with the flowering of the grass do not solely determine the distribution pattern.

Isolation of fungi from washed grass segments shows that certain of the fungi such as *Cladosporium*, *Alternaria*, *Epicoccum* and *Pleospora vagans* can be found in the lower internodes for periods of a year or more after flowering, when direct observation of fruit-bodies does not indicate their presence. Whilst it is possible that some of the culture records may result from spores on the surface of the segments which have not been removed by the washing process, this seems unlikely, and it seems probable that the mycelia of some of these fungi may persist long after conditions suitable for sporulation have passed. It is possible therefore that the picture of the distribution of the group 1 fungi is somewhat distorted by the use of sporulation as method of determining their presence, and that the problem resolves itself into determining the reasons for continued sporulation of these fungi on the upper internodes of grasses as compared with their short period of

sporulation on lower internodes. Webster (1957), in attempting to explain the difference in behaviour of the upper and lower internodes, has suggested that the group 1 fungi might demand for growth some substance present in decaying grass which, under the high humidity regime of the lower internodes, is more rapidly depleted there. It may be necessary to modify this hypothesis and restate it in terms of substances necessary for

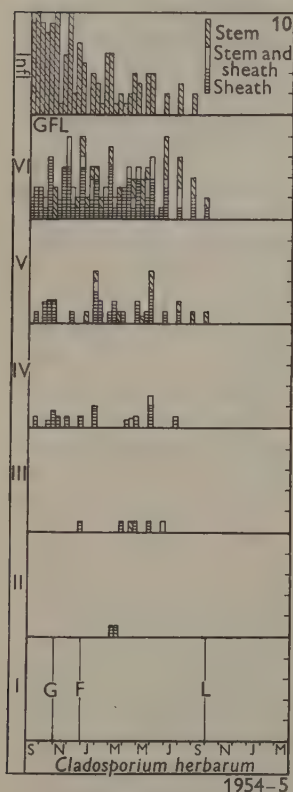


Fig. 3

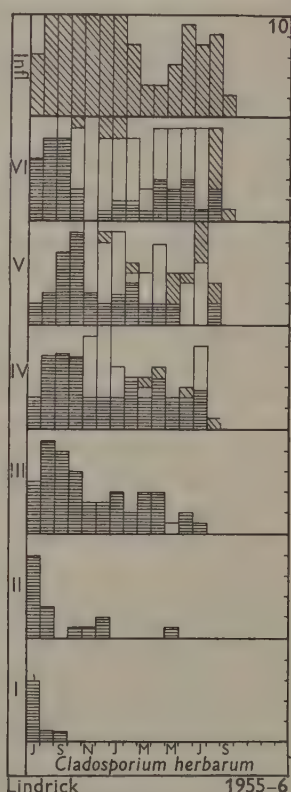


Fig. 4

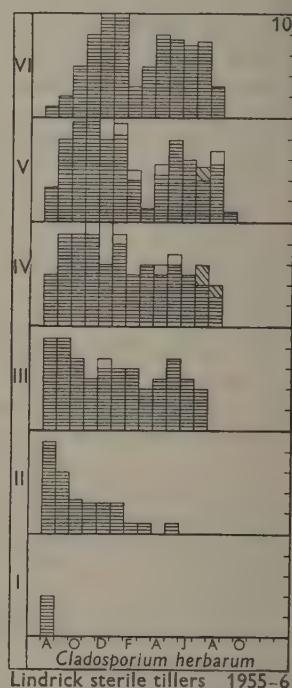


Fig. 5

Figs. 3-5. *Cladosporium herbarum*. Fig. 3. Frequency on monthly samples of 10 stems from each locality: Gardens, left-hand column; Fulwood, centre column; Lindrick, right-hand column. Fig. 4. Frequency on samples from Lindrick 1955-56 for comparison with Fig. 5 showing the frequency on sterile tillers during the same period.

sporulation rather than growth. Experimental work to support either form of the hypothesis is clearly needed. If the reasoning is correct, then one might expect group 1 fungi to persist on the lower internodes for longer periods under conditions of lower humidity. In an attempt to establish such conditions stems were laid horizontally on racks arranged 3 in. above the ground (i.e. under conditions comparable with those at internodes II and III), and 12 in. above the ground (i.e. under conditions comparable with those at internodes V-VI). Stems were placed on the racks in July, when the lower leaves were already colonized with group 1 fungi, and in

October. The colonization of the stems on the racks was compared with standing stems and with stems laid directly on the ground.

The results of these experiments were rather disappointing, and gave little support to the hypotheses outlined above. The lower internodes did not continue to support a high frequency of group 1 fungi in the sporulating condition, but sporulation continued on a high proportion of the upper internodes.

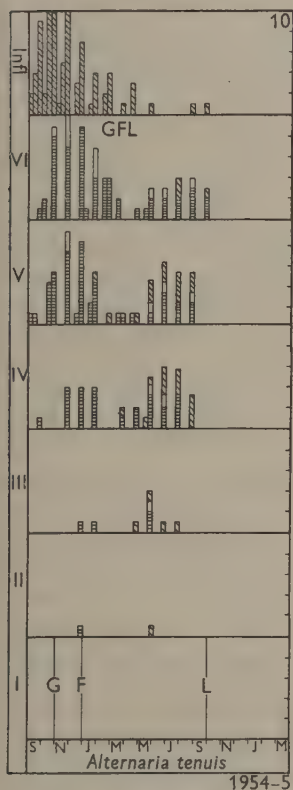


Fig. 6

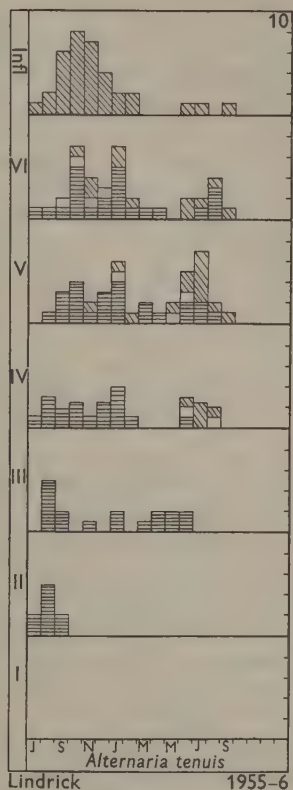


Fig. 7

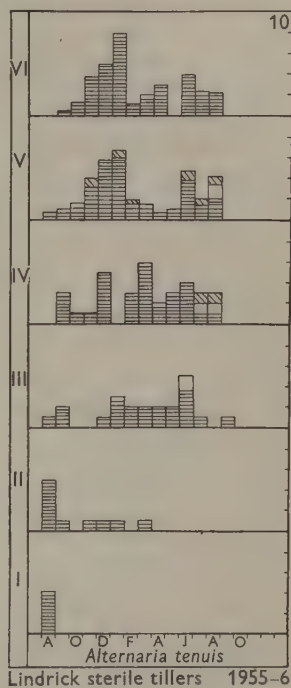


Fig. 8

Figs. 6-8. *Alternaria tenuis*. Fig. 6. Frequency on monthly samples of 10 stems from each locality. Note the higher frequency at Lindrick. Figs. 7 and 8 compare the frequency on flowering and sterile tillers at Lindrick 1955-56.

It is of particular interest here to consider some of the data available from surveys of the fungal spore content of the air. Hirst (1953) showed that *Cladosporium*, with *Alternaria*, is part of the dry-air spora with afternoon maxima. Richards (1956), by exposing Petri dishes at nine localities in Great Britain during April-October 1952, recorded about 70 species of fungi, of which *Cladosporium* was overwhelmingly predominant. *Cladosporium*, *Epicoccum*, *Botrytis*, *Alternaria* and *Pullularia* were relatively more important in rural areas than in cities. It is possible that much of this spore load comes from Gramineae, although these are very common

moulds on other substrates. The *Cladosporium* 'season' extended over June–September at all stations. *Cladosporium* formed 69.6 % of the entire catch followed by *Pullularia* at 5.5 %. The number of the former caught was directly related to the area of open country and land under crops, especially cereals, within a few miles of the stations. This peak or mould season may well coincide with the period of maximum fruiting of these primary colonizers on grasses. That the peak is early for *Agropyron* may be related to the fact that it is a later flowering grass than many, but even so abundant *Cladosporium* may be found on the leaves from May onwards.

Similar results have been obtained by Last (1955), and Hyde & Williams (1953), while Hyde & Williams (1946) showed that the greatest catch of *Alternaria tenuis* was during June to September, it being virtually absent during the late autumn and winter at Cardiff. It thus seems that the summer maxima of the fungi recorded on standing stems agreed with the time of their relative abundance as spores in the atmosphere.

Webster (1957) has drawn attention to the higher frequency of *A. tenuis* on samples of *Dactylis* collected from Lindrick. The same results were also noted with *Agropyron* (see Fig. 6). The reasons for this are not known; they may be due to differences in climate between the localities, differences in the host plant possibly related to differences in soil or to a greater spore concentration of this fungus at Lindrick, which is in a predominantly agricultural area. It would be of interest to elucidate the reasons underlying the phenomenon, especially in view of the implication of *Alternaria* spores as possible allergens (see Ainsworth, 1952).

Group 3. *Mollisia palustris*, *Leptosphaeria nigrans* and *Ophiobolus herpotrichus* show similar distributions on *Agropyron* (Figs. 9, 10). They fruit on the basal internodes of standing stems during April to November in the year following flowering. On collapsed stems, fruiting takes place during the same period but along the whole length of the stem in the first two. Occasional fruit-bodies are found during the summer on 2-year-old stems. The first two fungi are common on *Dactylis*, where they show analogous behaviour. *Pleospora trichostoma* has some similarities but may fruit higher on the stem, especially at internodes III and IV, and occasionally at internodes V and VI of standing stems (Figs. 12, 13). The fruiting of all these fungi is apparently stimulated by high-water content, and this is shown by their fruiting on the upper internodes of stems cut down during the previous autumn (Fig. 14). In all cases there is probably a delay period before fruit-bodies mature. Immature fruit bodies of *Leptosphaeria nigrans* and *Pleospora trichostoma* can often be found several months before ripe fruit-bodies. The position of these fungi in the succession may thus reflect particularly their requirements for sporulation, high humidity and possibly high temperature, and the time taken to produce fruit bodies.

On cut down stems the numbers of fruit bodies of these fungi are frequently much greater for the upper internodes than the lower. This has been taken to indicate differences in nutritional levels between upper and lower internodes.

Group 4. On *Agropyron*, *Tetraploa aristata* is not so frequent as on *Dactylis*, but both this fungus and *Helminthosporium hyalospermum* show similar patterns of distribution analogous to those on *Dactylis*. *Helminthosporium*

hyalospermum begins to fruit on basal internodes during the early winter, but its frequency increases during the following year (Fig. 11), and it is common along the whole length of old collapsed stems, especially in the Garden and at Fulwood. It has been collected only rarely at Lindrick. The distribution supports Webster's conclusions that this fungus sporulates most readily on old tissues with a high water content.

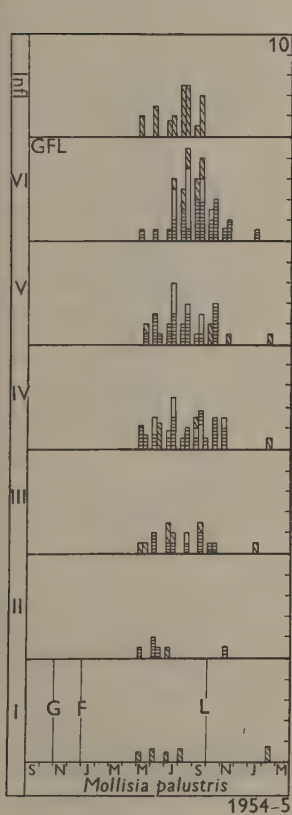


Fig. 9

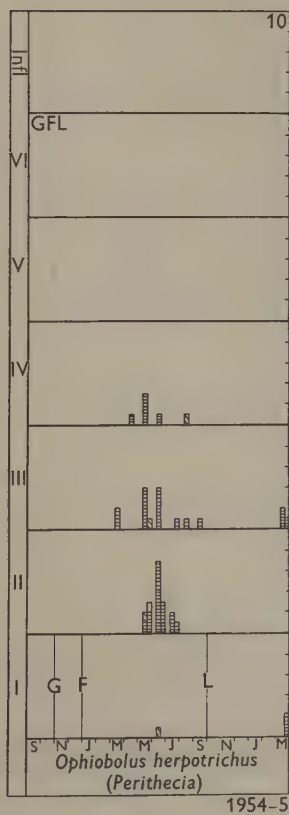


Fig. 10

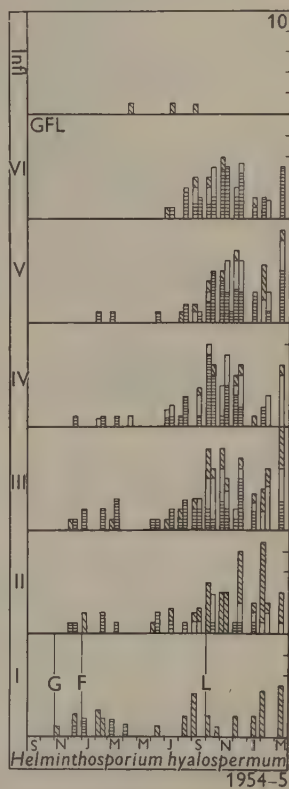


Fig. 11

Fig. 9. *Mollisia palustris*. Fig. 10. *Ophiobolus herpotrichus*. Fig. 11. *Helminthosporium hyalospermum*. All diagrams show the frequency of the fungi on monthly samples of ten stems from each locality.

Group 5. On *Agropyron*, although *Mycosphaerella recucita* and *Selenophoma donacis* have been collected, only *Selenophoma donacis* was common (Fig. 15). Although collected in all three localities it was most frequent at Lindrick on the upper internodes during the summer of the year following flowering. Its comparative rarity in the other localities may have been due to the earlier collapse of tillers there. There is evidence that the fruiting of this fungus takes place more frequently on stems of low-water content. For example, stems placed on racks 12 in. above the ground at the Gardens

in July bore the fungus from internodes III–VI in August, often with frequencies of 100 %, whilst [on] comparable samples at ground-level frequencies were very low.

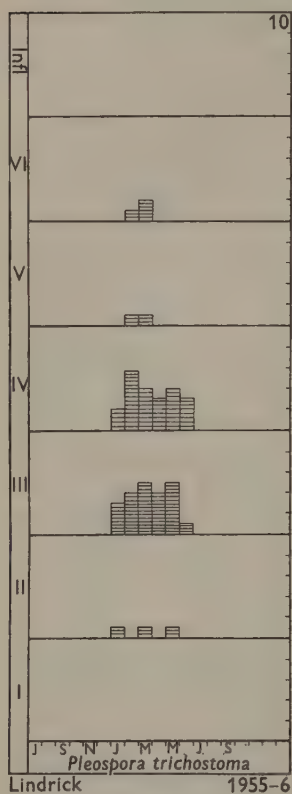


Fig. 12

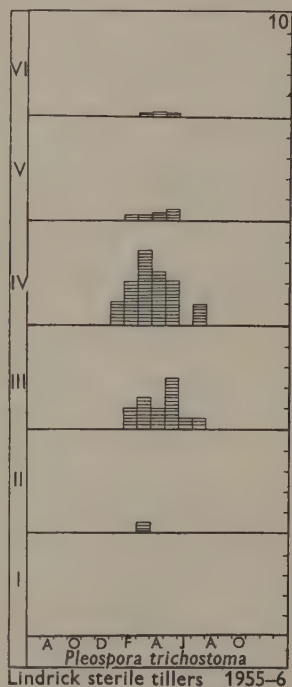


Fig. 13

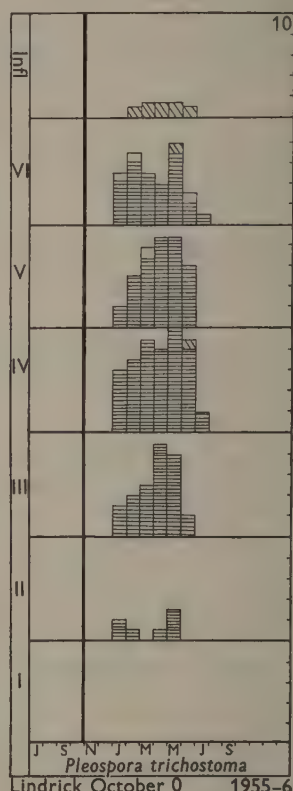


Fig. 14

Figs. 12–14. *Pleospora trichostoma*. Fig. 12. Frequency on monthly samples of flowering tillers from Lindrick 1955–56. Fig. 13. Frequency on monthly samples of sterile tillers from Lindrick 1955–56. Fig. 14. Frequency on flowering tillers from Lindrick cut down and laid on the ground in October 1956.

CULTURE STUDIES

The picture of the succession of fungi obtained by the direct observation method has been supplemented by isolations made from washed stems, and these have provided some additional information. For example, isolations from expanding inflorescence tillers show that *Pullularia pullulans* is frequently present on leaves even before they unfold, and was found during May to September on almost every washed segment. The distribution of the fungus as indicated by isolations from Lindrick, is shown in Fig. 16. It is interesting to note that quite a different picture is obtained by direct examination, for fruit-bodies of the fungus are rather inconspicuous and were only found on the upper internodes of standing stems during June to September in the year following flowering. Smit &

Wieringa (1953) have found *P. pullulans* on deciduous tree leaves soon after their appearance, and White (1945) has noted its association with *Cladosporium* and *Alternaria* on diseased flax.

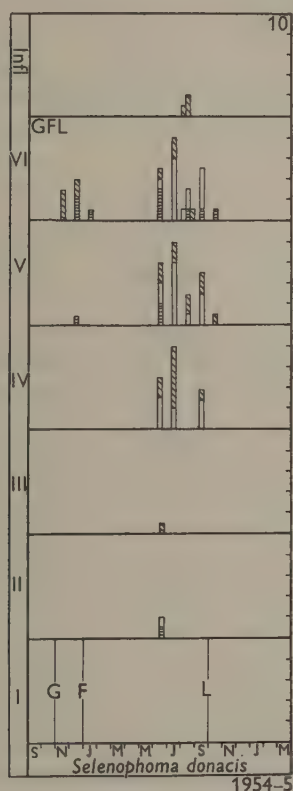


Fig. 15

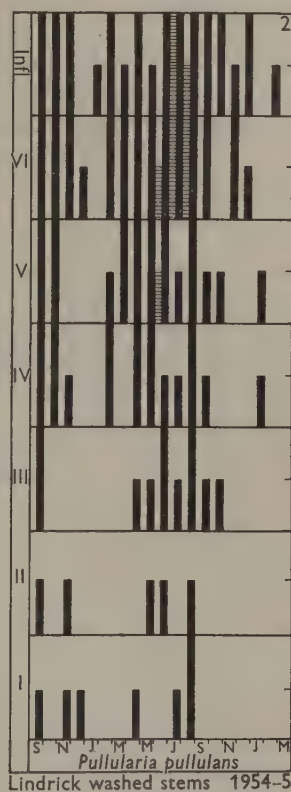


Fig. 16

Fig. 15. *Selenophoma donacis*. Frequency on monthly samples from each locality. Note the more frequent occurrence at Lindrick.

Fig. 16. *Pullularia pullulans*. Distribution as revealed by isolations from each segment of a monthly sample of 2 stems. (The solid black histograms indicate the presence of the fungus in culture, and the shaded histograms indicate that the fungus was observed directly on the stems before isolation.)

A number of other fungi are better represented by the culture method than by direct observation. For example, *Acrospermum graminum* has been isolated from the uppermost internodes of washed stems at Lindrick (see Webster, 1956*b*), but neither perithecia nor conidia were observed on the host during the present study.

Fungi in group 1 are particularly well represented in isolation studies since most of them grow rapidly and fruit readily in culture. It has already been shown that they can be isolated from levels at which they do not appear to sporulate. Thus in culture studies fungi of this group frequently predominate. If this were the only technique used, these fungi would

appear overwhelmingly important and the slower growing species or those that fruit less readily in culture from the basal internodes, would be missed and the picture of the succession would be one of greater abundance of the group 1 fungi.

It is of interest to note in view of the frequent records of Mucorales, *Penicillia* and *Aspergilli* from the soil, that in the present study these fungi were isolated very infrequently, even from old stems which had been lying on the soil surface for several months, and we conclude that during this phase of decay these organisms are relatively unimportant.

DISCUSSION

It is clear that the common fungi of *Dactylis* have a similar distribution on *Agropyron*, although there are a number of differences both in the species present and in the frequency of the common fungi on the two grasses. One of the most interesting problems raised by the two accounts of grass decay is the different course of succession in the three upper and the three lower internodes. Although the early stages of colonization of both these regions appear similar, at least so far as leaf blades and leaf sheaths are concerned (consisting mainly of fungi in group 1), the primary flora persists only on the upper internodes. On the lower internodes, under the more humid conditions prevailing, the primary flora quickly ceases to fruit, and fruit bodies of fungi in groups 3 and 4 take their place. Above, the primary flora may persist for over a year, and is augmented by *Selenophoma donacis* on standing stems.

The reasons for the different course of the succession may be a combination of the following factors:

(1) *Physical differences*, especially differences in the water content of the upper and lower internodes, are undoubtedly important, and high-water content appears to favour the development of fungi in groups 3 and 4. On the upper internodes only those fungi capable of resisting fluctuations in water content will persist, possibly in the form of resistant structures such as sclerotia, pycnidia or perithecia. Ability to grow under conditions where the air is frequently below saturation would also be advantageous in such a situation.

(2) *Nutritional differences*. Differences in the level of available nutrients at the time of senescence of the leaves seem likely (see Archbold, 1938; Nicol, 1936; Prat, 1948). The more intensive sporulation of groups 2 and 3 fungi on the upper internodes of cut-down stems has already been adduced as indirect evidence for these differences. It would be valuable to have a series of analyses from upper and lower internodes of grasses at various stages of decay or some other convincing demonstration of the nature of nutritional differences, and their effect on the growth and sporulation of some of the fungi concerned.

(3) *Differences in host resistance*. The rapidity of senescence of the upper internodes, compared with the lower ones has already been discussed. It seems possible that delayed senescence may hinder the sporulation of certain fungi. Anatomical differences between upper and lower internodes may be important in this connexion.

(4) *Competition*. It is probable that under the more humid conditions prevailing at the stem base, numerous fungi, bacteria and other organisms are capable of growth. It thus seems possible that the intensity of competition may in part explain the differences in colonization, particularly since more intensive microbiological activity might be expected to result in a rapid removal of nutrients from the substratum.

It is a pleasure to acknowledge help from various mycologists who have assisted in identification of fungi, but especially to Dr R. W. G. Dennis. We also wish to thank Mr N. L. Johnson for technical assistance. One of us (H. J. H.) is indebted to the Department of Scientific and Industrial Research for a maintenance grant.

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(Accepted for publication 3 July 1957)

TWO BIOLOGICAL RACES OF *UROMYCES* *LINEOLATUS* (DESM.) SCHROET.

By J. A. MACDONALD

Department of Botany, The University, St Andrews

(With Plate 7)

Two biological races of *Uromyces lineolatus* have been isolated from a community of *Scirpus maritimus* var. *compactus* near St Andrews, Scotland. They correspond with *U. l. f. sp. glaucis-scirpi* O. Jaap and *U. l. f. sp. scirpi-oenanthe-crocata* R. Maire. One race has its aecidial stage on *Glaux maritima* and the other on *Oenanthe crocata*. Neither race is capable of infecting the aecidial host of the other. They are not distinguishable from each other by the symptoms which they produce on *S. maritimus*. There are slight differences in the sizes of the spores produced on this host. These differences are statistically significant for the teleutospores. Uredospores formed on *Scirpus* following infection from either aecidial host can germinate in the following spring so that both biological races of the rust may be perpetuated on *Scirpus* without the intervention of the alternative hosts.

INTRODUCTION

Uromyces lineolatus (Desm.) Schroet. is dealt with by Guyot (1938, 1939, 1946, 1951, 1953). The uredo- and teleutospore stages of the rust occur commonly on *Scirpus maritimus*. They have been recorded on nine other species of the genus. The hosts of the spermagonial and aecidial stages include species belonging to nine genera of the Umbelliferae, *Glaux maritima* and *Hippuris vulgaris*. The fungus is widely distributed over all three continents in the northern hemisphere and occurs in South America (Guyot, *loc. cit.*) In Britain the rust has been recorded a number of times for England (Plowright, 1889; Grove, 1913; Grove & Chesters, 1934; Hora, 1956) and Scotland (Macdonald, 1939). The British hosts are given by Wilson & Bisby (1954) as: O, I, *Berula erecta*, *Glaux maritima*, *Oenanthe crocata*, *O. fistulosa*, *O. lachenalii*. II, III, *S. maritimus*. The suggestion has been made (Fort, 1940; Guyot, 1938; Jørstad, 1955) that there are biological races within this species, and that *Glaux maritima* acts as host for one which is distinct from the race or races occurring on Umbelliferae.

Fort (1940) published an account of work carried out in this Department on the rust as it was then known to occur in the St Andrews district of Fife. The habitat was a salt marsh to the south of the estuary of the River Eden, about 3 miles west of St Andrews, where field drainage produces patches of freshwater flushing. The spermagonial-aecidial host was *Oenanthe crocata* and the uredo-teleutospore host *Scirpus maritimus*.

We are indebted to Mr Nelves of the Royal Botanic Garden, Kew, for identifying material sent to him in 1952 as *S. maritimus* L. var. *compactus* (Hoffm.) Mayer. This seems to be the only variety present in the

St Andrews district. Guyot (1938) includes a record of the rust on *S. m.* var. *digynus*. Fort successfully carried out experimental infection of *Oenanthe crocata*, using material of *Scirpus maritimus* carrying teleutospores, and re-infected the latter species by means of the aecidiospores produced on *Oenanthe*. Her attempts to infect *Glaux maritima* in the same way were negative.

From 1938 to 1941 natural infections appeared on *Oenanthe* each June and *Glaux* remained healthy at the salt marsh. After the war observations were resumed on this rust population. The area was heavily grazed by cattle and in some years the greater part of the young growth of *Oenanthe* was eaten down about the time that aecidia are produced (June–July). In 1951 only one leaf bearing one group of immature aecidia was found. This was removed. In 1955 and 1956 cattle grazing removed virtually all foliage from mid-May till August. In the years 1952, 1953 and 1954 the plants were largely defoliated by attacks from the caterpillars of *Depressaria nervosa* Haworth during the June–July period. In these 5 years aecidia were not found on *Oenanthe*. The amount of the rust on *Scirpus maritimus* became very much reduced. In June 1951 aecidia were found on some plants of *Glaux maritima* growing on grassy tussocks above mud carrying *Scirpus*. The aecidial stage was found on *Glaux* each year from 1951 to 1956. Further work was undertaken in this department by Cammack (unpublished) to study the question of the existence of specialized races, to determine if there were morphological differences between populations on *Scirpus* originating from infection by aecidiospores from *Glaux* on one hand and *Oenanthe* on the other, to check on overwintering of uredospores discovered by Fort and to go further into details of the development and cytology of the fungus.

EXPERIMENTAL RESULTS

It was found that *Glaux maritima* could be experimentally infected with the rust when exposed to material of *Scirpus maritimus* bearing teleutospores collected at the salt marsh habitat. Further, *Oenanthe crocata* similarly treated remained free from infection. Fresh *Scirpus maritimus* was experimentally infected from aecidiospores formed on the *Glaux*. These findings raise several problems which may be stated as follows. Is the rust occurring on *Scirpus* uniform and is it able to infect *Oenanthe* and *Glaux* in different seasons because only one of the alternative hosts is available in a susceptible stage of growth at the time that germination of the teleutospores takes place? If this is so then the experiments carried out by Fort and Cammack must have unwittingly duplicated the correct conditions for one alternative host only. This seems improbable. If it is not so, can it be shown by isolation that the *Scirpus* carries two races or subspecies each with a separate aecidial host? Linked with these questions are the following points. Are there any size differences between the uredospores and teleutospores formed by infection of *Scirpus* from *Oenanthe* on the one hand and from *Glaux* on the other? Are the symptoms which infections from the two sources produce on *Scirpus* morphologically distinct?

In 1955 two other stations were found for the rust at Boarhills and at Tentsmuir on the other side of the Eden estuary about 7 and 4 miles

respectively from the salt marsh site. At Tentsmuir the same three host plants grow together very closely intermixed. The appropriate stages of the fungus occurred on all the hosts very freely in 1955, 1956 and 1957.

A third series of inoculation experiments was carried out in 1956 and 1957. Healthy plants of *Oenanthe crocata*, *Glaux maritima* and *Scirpus maritimus* were potted up. The *Scirpus* was kept in troughs containing several inches of rain water. Seeds of *Oenanthe* and *Glaux* were sown to provide seedlings for inoculation. Belljars $12 \times 7\frac{1}{2}$ in. with a bung hole closed with a rubber stopper were used as inoculating chambers. The inoculum of infected *Scirpus* material was placed on curtain netting stretched over glass or Perspex frames. A frame was suspended by string passing through the opening at the top of each belljar. The height of the frame was adjusted so that the lower side of the netting was just clear of the experimental plant when this was placed under the jar. The string was clamped by pressing home the bung. Each jar was placed in a closed propagating frame. The base was sealed by pressing the jar down into a layer of wet peat in the frame. After 24 hr. the bung was removed and the jar was raised slightly and lowered gently to change the air inside. After a further 24 hr. each plant was removed from its belljar and placed under a gauze-covered frame so that it was sheltered from direct sunlight. The frame was watered daily for 4 days, after which the plants were removed to an open, cool greenhouse with minimum temperature 40° F., for the rest of the experiment.

The infected *Scirpus* material used in the 1956 experiments was collected from the Tentsmuir site in October 1955 and kept outside over winter in a perforated tin so that the material did not dry out and did not become waterlogged. The *Scirpus* material used in the 1957 experiments was collected in both cases from artificially inoculated plants on 23 October 1956, and was stored in the same way. Samples of teleutospores were tested at weekly intervals from the beginning of March. As soon as the spores germinated freely after 24 hr. in tap water inoculation experiments were begun (10 April 1956, and 9 April 1957). Control plants of all three hosts have been maintained throughout the entire series of tests and have remained completely free from infection. Results obtained by Fort (1940), and Cammack (unpublished), for comparable experiments are noted under the appropriate tables.

In addition to the suspended frame method, positive results were given by placing infected *Scirpus* round the *Oenanthe* plants and by painting *Oenanthe* leaves with a suspension of teleutospores.

The results in Table 1 show that the sample of the fungus on *Scirpus* is capable of infecting both *Glaux* and *Oenanthe* in the same season. The experiments provided material on the two aecidial hosts from which attempts could be made to infect *Scirpus*.

From the experiments referred to in Table 2 material was available in 1957 to test the existence of *Glaux*- and *Oenanthe*-inhabiting races in the Tentsmuir population of the rust. The results obtained are summarized in Table 3.

The results given in Table 3 prove the existence on *Scirpus* of two biological races of *Uromyces lineolatus* with spermagonial and aecidial stages on

Table 1. *Inoculations from naturally infected Scirpus maritimus to Glaux maritima and Oenanthe crocata*

	No.	Date of inoculation	Date of infection	Time (days)
<i>To Glaux maritima</i>				
First inoculum	1	30 April	8 May	9
	2	2 May	13 May	12
	3	5 May	21 May	17
	4	8 May	Nil	—
Second inoculum	1	19 May	28 May	10
	2	21 May	1 June	11
	3	2 June	14 June	13
<i>To Oenanthe crocata</i>				
	1	12 April	28 April	17

Cammack in 1952 obtained infection on *Glaux* after 15 days, 1–14 March.

Natural infections were found on *Glaux* at Tentsmuir on 10 June.

Fort obtained positive results over a 3-month period from 23 February (18 days) to 20 May (6 days).

Natural infection was found on *Oenanthe* at Tentsmuir on 27 May.

Table 2. *Inoculations from artificially infected Glaux and Oenanthe on to Scirpus*

No.	Date of inoculation	Date of infection	Time (days)
<i>From Glaux maritima</i>			
1	15 May	Nil	—
2	10 June	Nil	—
3	11 July	23 July	13
<i>From Oenanthe crocata</i>			
1	11 June	6 July	26

Infected *Glaux* plants planted in *Scirpus* pots. *Scirpus* leaves brushed with acidiospore suspension.

The infection obtained from *Glaux* was very light. It was increased by artificially spreading uredospores when the uredosori opened.

Infected *Oenanthe* plants placed with *Scirpus* pots. *Scirpus* leaves brushed with acidiospore suspension.

Fort also infected *Scirpus* from *Oenanthe* but could not estimate the time taken for the infection to develop due to the experimental method employed.

Natural infection was found on *Scirpus* at Tentsmuir on 24 July.

either *Glaux maritima* or *Oenanthe crocata*, each race being unable to infect the other alternative host.

As in 1956, *Scirpus* was reinfected from *Glaux* and from *Oenanthe*. Again difficulty was experienced in obtaining infection from *Glaux*. No infection was produced by placing infected plants among well-grown plants from 13 May onwards. When it became clear that this method had failed, limited infection was produced by suspending net-covered frames above the trough containing the *Scirpus* plants and prolonging the life of the *Glaux* inoculum by spraying the gauze daily. Infection became obvious after 17 days (15–31 July).

From 13 May onwards all infected *Oenanthe* plants were put beside *Scirpus*, which became freely infected by 26 June.

Table 3. *Inoculations of Glaux and Oenanthe from Scirpus artificially infected in 1956*

	No.	Date of inoculation	Date of infection	Time (days)
From <i>Scirpus</i> infected from <i>Glaux</i>				
First inoculum	1	<i>Gl.</i> 13 April	26 April	14
		<i>Oen.</i> 13 April	No infection	—
	2	<i>Gl.</i> 15 April	26 April	12
		<i>Oen.</i> 15 April	No infection	—
	3	<i>Gl.</i> 17 April	30 April	14
		<i>Oen.</i> 17 April	No infection	—
Second inoculum	1	<i>Gl.</i> 3 May	15 May	13
		<i>Oen.</i> 3 May	No infection	—
Third inoculum	1	<i>Gl.</i> 15 May	30 May	16
		<i>Oen.</i> 15 May	No infection	—
Fourth inoculum	1	<i>Gl.</i> 3 June	No infection	—
		<i>Oen.</i> 3 June	No infection	—
From <i>Scirpus</i> infected from <i>Oenanthe</i>				
First inoculum	1	<i>Gl.</i> 20 April	No infection	—
		<i>Oen.</i> 20 April	30 April	11
	2	<i>Gl.</i> 22 April	No infection	—
		<i>Oen.</i> 22 April	30 April	9
	3	<i>Gl.</i> 24 April	No infection	—
		<i>Oen.</i> 24 April	3 May	10
Second inoculum	1	<i>Gl.</i> 10 May	No infection	—
		<i>Oen.</i> 10 May	19 May	10
Third inoculum	1	<i>Gl.</i> 24 May	No infection	—
		<i>Oen.</i> 24 May	6 June	14
Fourth inoculum	1	<i>Gl.</i> 26 June	No infection	—
		<i>Oen.</i> 26 June	No infection	—

Natural infections were found on *Glaux* at Tentsmuir on 17 June. Pots of *Oenanthe* seedlings inoculated from the first inoculum on 22 April, from the second inoculum on 10 May and from the third inoculum on 24 May became very heavily infected after 11, 10 and 11 days respectively.

Natural infection of *Oenanthe* was found at Tentsmuir on 17 June and at the Salt Marsh on 2 July.

Natural infection of *Scirpus* was found at Tentsmuir on 7 July.

There is no perennial infection on any of the three hosts. Plants infected one season remain free from infection in subsequent years. Anatomical examination has shown that the mycelium of the rust is restricted to the vicinity of the erumpent spermatogonia, aecidia, uredo- or teleutosori. Infected *Glaux* stems are very swollen and often distorted (Plate 7), infected *Oenanthe* stems may be distorted also, though the fungus is more usually confined to the leaves.

The figures quoted show that discharge of basidiospores may go on for at least a week from one lot of infected *Scirpus* (Table 1).

April to May is the period over which the aecidial hosts are most readily infected. It was possible to infect *Glaux* experimentally for at least 34 days in 1956 (Table 1) and 32 days in 1957 (Table 3). *Oenanthe* was experimentally infected over a period of 34 days in 1957 (Table 3). These

periods largely coincide. From these figures and the extraordinary figure of at least 84 days obtained by Fort, it is clear that in any season both *Glaux* and *Oenanthe* are available for infection in a wide range of stages of growth at the time that basidiospores are discharged, and both may produce aecidiospores to perpetuate on *Scirpus* their races of the rust. *Scirpus* was most readily infected during June-July. Because of their size, *Scirpus* plants could not be put under belljars for inoculation. The aecidial hosts were placed among the *Scirpus* plants growing in the troughs. By this method the large *Oenanthe* plants were brought into intimate contact with the leaves of the *Scirpus* and heavy infections were produced. With *Glaux* very light infection only was obtained until aecidium-producing material was suspended above the *Scirpus* on a net-covered frame. Even then the infection obtained was fairly light. Cammack found that the teleutospores were not deciduous. This links up with the observation that, at the Eden estuary sites, *Glaux* is infected mainly when it grows on tussocks above the mud in which the *Scirpus* is growing, so that basidiospores from *Scirpus* leaves of the previous season can fall on to the developing *Glaux* and the aecidiospores in their turn are produced very close to the developing *Scirpus* leaves as they grow up past the *Glaux*. All infected *Glaux* found so far is within 9 ft. of infected *Scirpus*. Infected *Oenanthe* occurs up to 30 ft. away from the infected *Scirpus*.

The ability of uredospores to germinate over winter was recorded by Fort (1940). Confirmatory tests were carried out at 20° C. each month from September to April 1951-2, using 0.5 % sucrose solution in distilled water (Table 4). Though there was a rapid drop in the percentage and rapidity of germination between October and November and again between November and December, 2 % germination was obtained even in April. By this time new growth had appeared on the *Scirpus* plants. The rust can therefore survive from year to year, perpetuating itself on *Scirpus* by means of uredospores, to the exclusion of the aecidial hosts.

Table 4

Month	Germination of uredospores	Time (hr.)	Germination (%)
September	+	24	100
October	+	24	100
November	+	24	60
December	+	72	5
January	+	72	5
February	+	72	2
March	-	72	0
April	+	72	2

There is no obvious morphological difference between the sori formed on *Scirpus* as a result of infection from *Glaux* and those formed after infection from *Oenanthe*. The appearance of the spores is also the same. Uredo- and teleutospores formed on *Scirpus* following infection from *Glaux* were measured and the measurements compared with those given by Fort (1940) for her *Oenanthe-Scirpus* material.

Small samples of teleutospores were measured at fortnightly intervals from the beginning of October. When the value of the average measure-

ments became constant it was assumed that the spores had become fully developed. The range of measurements obtained (uredospores $21.5-34.6 \times 16.3-25.1 \mu$; teleutospores $25.9-45.3 \times 15.0-23.8 \mu$) agrees almost exactly with the figures given by Grove (1913) who was also dealing with *Glaux-Scirpus* material, and differs slightly from Fort's figures. These slight differences were analysed statistically employing methods recommended by Loomis & Schull (1937). The differences between the two sets of uredospores are not significant, the differences between the two sets of teleutospores are. Those for the breadths show a high order of significance. Nevertheless, the differences are not such that they can be seen by inspection and they are therefore of doubtful practical value in distinguishing between the races. Fort (1940) recorded minute protuberances on the outside of the wall of the uredospores in her *Oenanthe-Scirpus* material. Cammack (unpublished), found that the walls of the uredospores in his *Glaux-Scirpus* material were completely smooth. Uredospores experimentally produced by infection of *Scirpus* from both aecidial hosts in 1957 were therefore examined. It was found that there were minute echinulations on the walls of uredospores from both sources. These are much more easily seen in the immature spores. Slight irregularities in the outside of the uredospore wall are present in both cases also.

The two biological races isolated agree with those recorded by Guyot (1938) as *Uromyces lineolatus* (Desm.) Schroet. f. sp. *glaucis-scirpi* O. Jaap, and *U. l.* f. sp. *scirpi-oenanthe-crocata* R. Maire.

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EXPLANATION OF PLATE 7

Plants of *Glaux maritima* infected by *Uromyces lineolatus* showing aecidia on swollen and distorted stems. $\times 3$.

(Accepted for publication 27 August 1957)

The Society gratefully acknowledges a grant towards the cost of illustrating this paper from the University of St Andrews.



Trans. Brit. mycol. Soc. **41** (2), 185-195 (1958).

LEAF LITTER FUNGI FOUND ON *CAREX* *PANICULATA* L.*

By G. J. F. PUGH

Department of Botany, The University, Nottingham

(With 4 Text-figures)

The distribution of fungi growing on *Carex paniculata* has been studied by the identification of species on material collected at intervals during each year, and by the isolation of species from surface-sterilized leaf tissues. Changes in the fungal flora as decomposition proceeds have been recorded. The commonest species isolated from surface-sterilized leaves were *Cephalosporium acremonium*, *Cladosporium herbarum*, *Fusarium culmorum*, species of *Mucor* and of *Penicillium*, and *Trichoderma viride*. The most frequently occurring species on collected leaf litter were *Metasphaeria cumana* and *Stysanus stemonites*. Species of Dematiaceae and Stilbaceae were commoner as superficial sporophores than were species of the Moniliaceae.

Isolations were more numerous during the summer months. This is correlated with the higher temperatures and lower water content of the leaf tissues.

INTRODUCTION

While certain aspects of the ecology of fungi have received some detailed consideration, there are few ecological observations of the fungi developing on herbaceous leaf litter. The tussocks of *Carex paniculata* provide an extremely favourable habitat for the study of all stages in the colonization of moribund and dead leaf tissues, as much because a single plant species contributes to the litter as because the bulk of this litter has accumulated over successive years.

MATERIALS AND METHODS

C. paniculata is locally abundant in Oxtun woods, Nottinghamshire (National Grid Reference 43/618508), and easily available for making regular collections. Individual plants have a core composed of closely packed decaying leaf bases, penetrated in all directions by living and dead roots. The current year's leaves grow from the crown of this core, attaining a length of about 2 m. at maturity. These leaves die back from the distal ends while still actively growing from the basal meristems; thus the distal ends may be dead, and lying on the ground, while the proximal ends are still healthy. This die-back is progressive and slow, eventually involving the whole leaf. At first, the dead leaf blades hang from the crown, but later become detached and form a mass of leaf debris around the base of the core. The regions shown in Fig. 1 were used to provide an arbitrary basis for separating the leaf litter into zones of increasing decomposition.

* Part of a thesis submitted for the degree of Ph.D. in the University of Nottingham.

Collections were arranged in the following categories (the numbers refer to Fig. 1): 1 = recently dead leaves, 2 = leaves formed in previous years and lying around the tussock, 3 = old leaf base material forming the bulk of each tussock core. In the text these categories will be referred to as outer leaves (1), inner leaves (2) and interior of the tussock (3).

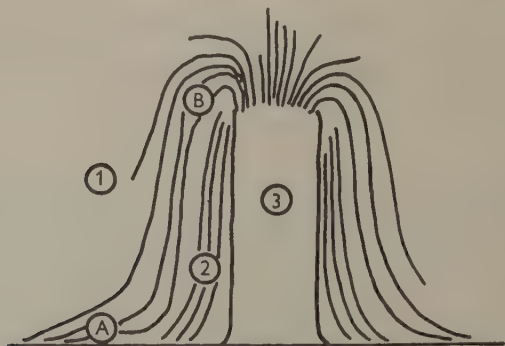


Fig. 1. Diagrammatic vertical section through a tussock of *Carex paniculata*. The numbers are explained in the text.

At each collection, portions of leaves from each category were placed in sterile tubes. Outer leaves, which were dead through out their length, were sampled from a distal area, about 20 cm. from the leaf tip (1 A), and from a proximal area, about 20 cm. from the leaf base (1 B). Inner leaves were sampled at random. Material from the interior of the tussocks was collected with sterile forceps and scalpel, after removing the superficial debris. A set of similar samples was collected in air-tight containers for the determination of pH and water content. In this set, the middle portion of the outer leaves was sampled. The temperature of each zone was recorded at the time of collection.

Leaf samples were examined under a binocular dissecting microscope as soon after collection as possible, and sporulating fungi were recorded. Other samples were incubated at room temperature in moist chambers (Keyworth, 1951) and species developing on such leaves were identified.

Small samples, 1 cm. long, cut from the leaves, were surface-sterilized, washed, dried and plated-out, four samples per plate, on each of the following media: *Carex* extract, Czapek-yeast, and potato-dextrose agars with and without 1:15,000 Rose Bengal (Smith & Dawson, 1944). Rose Bengal reduced the number of isolations and the number of species per leaf portion, and with very decomposed material inhibited all fungal growth. This contradicts the observations of Dawson & Dawson (1947). Mercuric chloride at 1:1000, as used by Davies (1935) and Walker (1941), was allowed to act for 60 sec. on outer leaf material, for 30 sec. on inner leaf material, and for 15 sec. on the decomposed material from the interior of the tussock. Material was also surface-sterilized in silver nitrate, using similar times of immersion. The silver nitrate was inactivated by saturated sodium chloride before the material was washed, dried and plated-out.

Hydrogen-ion concentrations were determined on 5 g. samples of the different collection categories, extracted in 20 ml. distilled water by constant shaking for 1 hr. A Cambridge glass electrode potentiometer was used to measure the pH of the decanted liquid. The water content of samples from each of the collection categories was determined by weighing the freshly collected samples, and reweighing after drying to constant value at 105° C. The results were calculated as percentage dry weight.

DISTRIBUTION OF THE FUNGI

On freshly collected leaf litter

The species observed on freshly collected leaf litter are listed in Table 1. The majority of the species occurred in the outer leaves, and members of the Ascomycetes, Basidiomycetes and Sphaeropsidales were almost entirely

Table 1. *Distribution of species observed on freshly collected leaf litter*

a. = abundant; o.a. = occasionally abundant; c. = common; o. = occasional; r. = rare.

	Outer leaves	Inner leaves	Interior of tussock
<i>Acremoniella verrucosa</i> Togn.	o.	—	—
<i>Aschochyta sodalis</i> Grove	o.	—	—
<i>Coniothyrium</i> sp.	r.	—	—
<i>Cryptoderis caricina</i> Rehm	o.a.	—	—
<i>Didymella</i> sp.	o.	—	—
<i>Diplodia herbarum</i> Lev.	o.	—	—
<i>Didymosphaeria palustris</i> (Berk. & Br.) Sacc.	r.	—	—
<i>Leptosphaeria? gigaspora</i> Niessl	c.	—	—
<i>L. microscopica</i> Karst	c.	—	—
<i>L.? plantuscula</i> (Riess) Wint.	c.	—	—
<i>Metasphaeria cumana</i> (Sacc. & Speg.) Sacc.	a.	—	—
<i>M. rimularum</i> (Cooke) Sacc.	o.	—	—
<i>Puccinia caricis</i> Reb.	a.	—	—
<i>Pycnothyrium junci</i> Grove	o.a.	—	—
<i>Stagonospora paludosa</i> Sacc.	c.	—	—
<i>Aspergillus niger</i> van Tieghem	—	r.	—
<i>Acrothecium simplex</i> Berk. & Br.	o.	o.	—
<i>Alternaria tenuis</i> Nees ex Wallr.	o.	o.	—
<i>Diplodina caricis</i> Grove	c.	r.	—
<i>Nectria inventa</i> Peth. (conidia)	r.	o.	—
<i>Niptera dilutella</i> Fr.	o.a.	o.	—
<i>Periconia paludosa</i> Mason & Ellis	c.	o.	—
<i>Sporocybe rhopaloides</i> Sacc. & Roum.	r.	o.	—
<i>Stysanus stemonites</i> (Fr.) Corda	a.	o.	—
<i>Cladosporium herbarum</i> Fr.	o.	c.	o.
<i>Helminthosporium biforme</i> Mason & Hughes	o.	o.	o.
<i>Mucor hiemalis</i> Wehmer	o.	o.	o.
<i>Penicillium</i> sp.	r.	o.	o.
<i>Tetraploa aristata</i> Berk. & Br.	c.	c.	c.
<i>Trichoderma viride</i> Fr.	o.	o.	c.

confined to this habitat. *Cryptoderis caricina* was found in the bases of recently dead leaves; a species of *Didymella* was often seen in the comparatively dry blades, associated with species of *Leptosphaeria* and *Metasphaeria*. *Didymosphaeria palustris* was collected twice, on the fairly decomposed distal portions of outer leaves. The species of *Leptosphaeria* and *Metasphaeria*

were all found in the drier parts of the outer leaves. *M. cumana* was the most frequently collected member of the Pyrenomycetes; rows of its perithecia occurred commonly between vascular bundles.

Puccinia caricis was collected regularly throughout the summer on all living leaves. Old sori could be recognized on dead leaves, and it was noticeable that areas which had a heavy rust infection carried few pyrenomycetes.

Members of the Sphaeropsidales were normally confined to the leaf blades, but *Pycnothyrium junci* was found on leaf bases.

Members of the Moniliales were more evenly distributed between the different leaf categories. *Stysanus stemonites* was one of the species most commonly collected in the field, where it was abundant on comparatively dry leaf litter, and *Cladosporium herbarum*, *Periconia paludosa*, *Tetraploa aristata* and *Trichoderma viride* were all frequently encountered.

On leaf litter incubated in moist chambers

Species developing on incubated leaves are listed in Table 2. *Chaetomium globosum* and *Sordaria macrospora* produced superficial perithecia, the latter species being especially abundant if free water was present in the

Table 2. *Distribution of species observed on leaf litter incubated in moist chambers*

a. = abundant; o.a. = occasionally abundant; c. = common; o. = occasional; r. = rare.

	Outer leaves	Inner leaves	Interior of tussock
<i>Acremoniella verrucosa</i> Togn.	o.	—	—
<i>Fusarium culmorum</i> (Smith) Sacc.	o.	—	—
<i>Periconia paludosa</i> Mason & Ellis	o.	—	—
<i>Nectria inventa</i> Peth. (conidia)	—	o.a.	—
<i>Niptera dilutella</i> Fr.	—	r.	—
<i>Acrothecium simplex</i> Berk. & Br.	o.	o.	—
<i>Alternaria tenuis</i> Nees ex Wallr.	o.	o.	—
<i>Cephalosporium acremonium</i> Corda	c.	o.	—
<i>Chaetomium globosum</i> Fr.	o.a.	o.	—
<i>Sordaria macrospora</i> Auersw.	o.a.	o.	—
<i>Sporocybe rhopaloides</i> Sacc. & Roum.	r.	o.	—
<i>Stysanus stemonites</i> (Fr.) Corda	o.a.	o.	—
<i>Cladosporium herbarum</i> Fr.	c.	c.	o.
<i>Helminthosporium biforme</i> Mason & Hughes	o.	o.	o.
<i>Mucor hiemalis</i> Wehmer	o.	o.	c.
<i>Penicillium</i> sp.	o.	o.	o.
<i>Tetraploa aristata</i> Berk. & Br.	o.	c.	o.
<i>Tilachladium</i> sp.	o.	o.	r.
<i>Trichoderma viride</i> Fr.	c.	c.	c.

moist chamber. Both species were of sporadic occurrence, as also was *Stysanus stemonites*, which only became abundant if the incubation chambers were allowed to dry out. *Cephalosporium acremonium*, *Cladosporium herbarum*, *Tetraploa aristata* and *Trichoderma viride* were of much more constant occurrence, being present on some leaf portions in most collections.

In surface-sterilized moribund leaves

A number of moribund leaves which were rapidly dying back from the apex were collected in August 1953, and samples were cut from the green, from the brown, and from the yellow transition zones. These leaf samples were surface-sterilized and plated-out. The distribution of the species isolated from these portions is given in Table 3. In the green parts of the

Table 3. *Distribution of species in moribund and recently dead leaf tissues, given as number of isolations from 60 leaf portions in each category*

	Brown	Yellow	Green
<i>Penicillium</i> spp.	45	—	—
<i>Verticillium effusum</i> Otth.	3	—	—
<i>Mucor</i> spp.	2	—	—
<i>Trichoderma viride</i> Fr.	1	—	—
<i>Fusarium culmorum</i> (Smith) Sacc.	40	29	16
<i>Cephalosporium acremonium</i> Corda	3	5	9
Sterile mycelium	2	5	6
<i>Cladosporium herbarium</i> Fr.	3	1	2
<i>Tilachladium</i> sp.	2	1	3
<i>Sporocybe rhopaloides</i> Sacc. & Roum.	—	5	—
<i>Fusarium?</i> <i>solani</i> (Mart.) Appel & Woll.	—	1	—
<i>Sordaria macrospora</i> Auersw.	—	1	—
<i>Metasphaeria mycelium</i>	—	13	16
<i>Botrytis cinerea</i> Fr.	—	2	2
<i>Alternaria tenuis</i> Nees ex Wall.	—	1	2
<i>Acrothecium simplex</i> Berk. & Br.	—	1	1
<i>Cylindrocarpum radiculicola</i> Woll.	—	1	1

leaves, *Fusarium culmorum* and '*Metasphaeria mycelium*' were isolated most frequently. The latter was a sterile grey mycelium, similar in cultural characteristics to that obtained by germinating ascospores of *M. cumana*. While the number of isolations of *F. culmorum* gives the impression that this species invades the green tissues from previously colonized brown tissues, so, on the same basis, '*Metasphaeria mycelium*' appeared concentrated in the green and the transition zones. Species of *Penicillium*, which were abundant in the dead tissues, were not isolated from the moribund parts of the leaves.

In addition to these isolations, perithecia of *Metasphaeria cumana* were often found in the brown zone within 2–3 cm. of the green parts of the leaf. It is suggested that *Fusarium culmorum* and *Metasphaeria cumana* may be weak parasites on the moribund portions of the leaves.

In surface-sterilized leaf litter

The number of species isolated was greatest in the outer leaves, while the inner leaves and the interior of the tussock showed a progressive decline in the number of species. Similarly, the number of isolations was greatest from the distal ends of the outer leaves, and was least from the interior of the tussock (Table 4).

The larger number of isolations from the distal ends of the outer leaves, as compared with those from the proximal ends, may be correlated with the more uniform microclimate at the surface of the soil. The differences

between numbers of isolations from the distal ends of the outer leaves and those from the inner leaves, are thought to be a result of the differing nutritional status of the two leaf categories.

Table 4. *Distribution of species isolated on agar from surface-sterilized leaf portions, given as number of isolations from 448 portions in each leaf category*

	Outer leaves		Inner leaves	Interior of tussock
	Distal ends	Proximal ends		
<i>Pullularia pullulans</i> (de Bary) Berkh.	22	18	—	—
<i>Stagonospora paludosa</i> Sacc.	5	4	—	—
<i>Aspergillus niger</i> van Tieghem	3	2	—	—
<i>Phoma</i> spp.	51	40	5	—
Sterile mycelium, white	14	27	24	—
<i>Alternaria tenuis</i> Nees ex Wallr.	6	6	21	—
<i>Botrytis cinerea</i> Fr.	7	7	15	—
<i>Fusarium culmorum</i> (Smith) Sacc.	237	115	52	2
<i>Cladosporium herbarum</i> Fr.	185	83	77	26
<i>Cephalosporium acremonium</i> Corda	111	38	34	10
<i>Mucor hiemalis</i> Wehmer	38	35	23	20
<i>Verticillium effusum</i> Otth.	12	20	13	14
<i>Tilachladium</i> sp.	19	40	9	5
Sterile mycelium, brown	19	9	1	3
<i>Penicillium</i> spp.	205	265	397	303
<i>Mucor ramannianus</i> Moeller	19	22	69	14
<i>Trichoderma viride</i> Fr.	111	82	87	132
<i>Mortierella</i> spp.	27	10	6	16

Table 5. *Distribution of species of Penicillium, given as number of isolations from 448 portions in each leaf category*

	Outer leaves		Inner leaves	Interior of tussock
	Distal ends	Proximal ends		
<i>Penicillium spinulosum</i> Thom	128	140	196	56
<i>P. citrinum</i> Thom	—	48	94	41
<i>P. thomii</i> Maire	—	13	20	—
<i>P. notatum</i> Westling	11	—	14	88
<i>P. corylophilum</i> Dierckx	26	20	34	73
<i>P. aurantio candidum</i> Dierckx	—	17	5	22
<i>P. chrysogenum</i> Thom	32	2	34	14
<i>P. frequentans</i> series	8	14	—	7
Other species	—	11	—	2
	205	265	397	303

Of the species limited to the outer leaves, *Pullularia pullulans* was most prolific, while species of *Phoma* were almost confined to this leaf category. *Alternaria tenuis* and *Botrytis cinerea* were commoner in the inner leaves, but were not isolated from the interior of the tussock. Other species were isolated from all leaf categories. The number of isolations of several of these species decreased towards the interior of the tussock. The most striking examples are afforded by *Fusarium culmorum*, *Cladosporium herbarum* and *Cephalosporium acremonium*. Other species, notably species of *Penicillium* and *Mucor ramannianus*, were more frequent in the inner leaves. The distribution of *Trichoderma viride* was unusual in that almost as many

isolations were obtained from the distal ends of the outer leaves as from the interior of the tussock, while the numbers from the other two leaf categories were much lower. When individual species of *Penicillium* are considered (Table 5), it can be seen that several were more abundant in a particular type of leaf litter. Most isolations were made from the inner leaves, where *P. spinulosum* was the commonest species. This species was also very frequent in the outer leaves. *P. citrinum* had a somewhat similar distribution, but with fewer isolations, and it was absent from the distal ends of the outer leaves. *P. notatum* and *P. corylophilum* were the only species throughout this work which were much more common in the interior of the tussock than elsewhere.

SEASONAL VARIATIONS

It is appropriate to consider the results of the isolation experiments in relation to certain environmental factors, such as rainfall (measured at Nottingham University, 9-10 miles from Oxton woods), and water content,

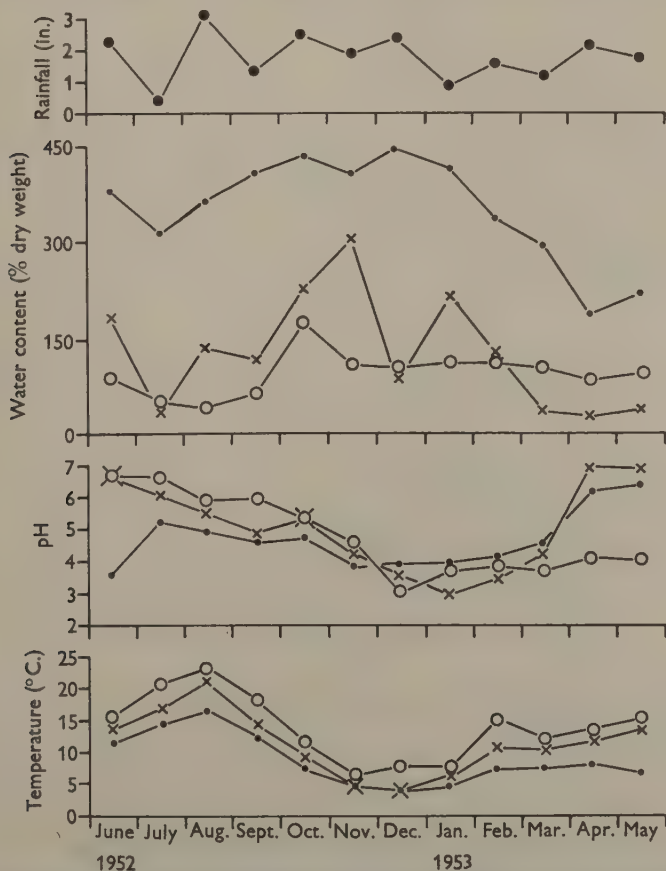


Fig. 2. Physical conditions in the microhabitat. ●—●, Rainfall; ○—○, outer leaves; x—x, inner leaves; ●—●, interior of tussock.

temperature and pH of the microhabitat (Fig. 2). Water content of the tissues, although constantly very high in the interior of the tussock, tended to be seasonal, being highest in winter. The temperature and pH curves are somewhat similar to each other, being lower in winter and higher in summer, so that they are approximately inverse to that for water content.

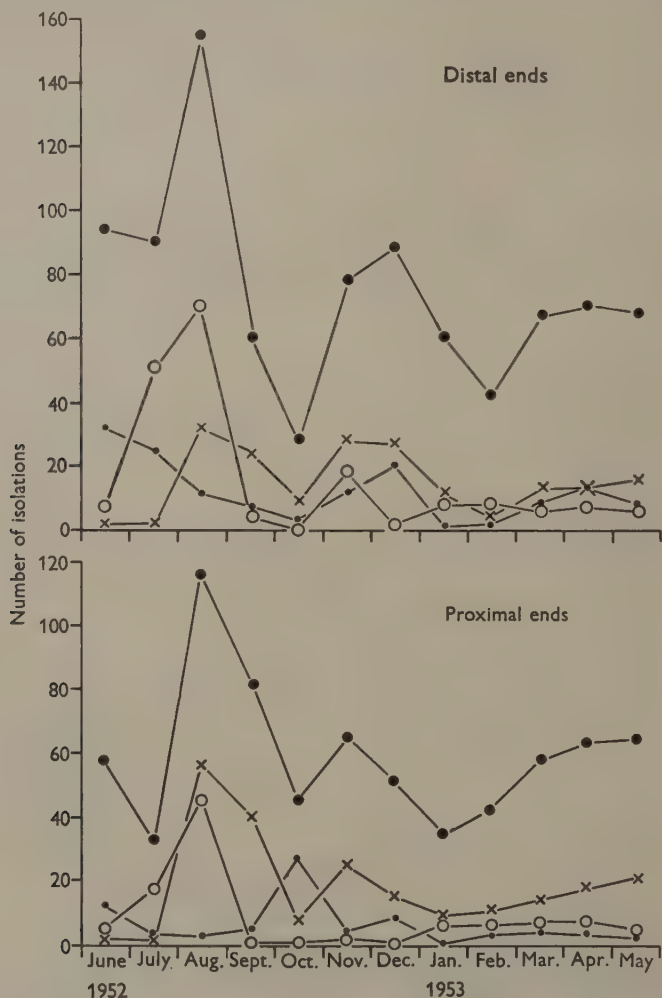


Fig. 3. Seasonal fluctuations in the number of isolations from the outer leaves. ●—●, Total isolations; •—•, *Cladosporium herbarum*; ○—○, *Fusarium culmorum*; x—x, species of *Penicillium*.

The total number of isolations for each leaf category, and the number of isolations of the more prominent species are shown in Figs. 3 and 4. The isolations made from both ends of the outer leaves were highest in August, and these peaks are related to a sudden increase in the isolations of *Fusarium culmorum* and of species of *Penicillium*. Thereafter *Fusarium culmorum* equally

suddenly decreased in numbers, in contrast to species of *Penicillium*, which decreased more slowly, so that some were isolated at each collection. *Cladosporium herbarum* was most active at the distal ends of the outer leaves in June, with a smaller peak in December, while at the proximal ends, this species was most active in October. In the inner leaves there was no

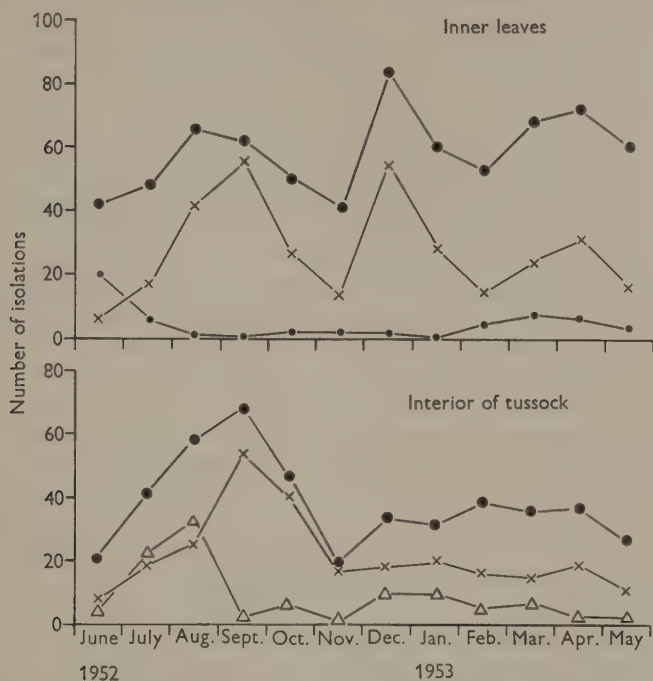


Fig. 4. Seasonal fluctuations in the number of isolations from the inner leaves and the interior of the tussock. ●—●, Total isolations; ●•, *Cladosporium herbarum*; x—x, species of *Penicillium*; △—△, *Trichoderma viride*.

absolute peak of isolations such as occurred in the outer leaves, but there were fluctuating high and low values which were almost entirely caused by species of *Penicillium*. These species also provided the bulk of the September peak of isolations from the interior of the tussock. *Trichoderma viride* was more or less constant after a period of increased activity in July and August.

DISCUSSION

The fungal flora of the dead leaves of *Carex paniculata* altered as the leaf material decomposed. The number of species, and the number of isolations, was least in the oldest litter, and greatest in the newly dead leaves. Chesters (1949) has postulated that the so called 'soil fungi' in fact grow upon particles of organic matter, so it may be appropriate to consider some opinions regarding the changes which take place during colonisation of such particles in the soil. Garrett (1951) attributed changes in the flora to the progressive breakdown of the newly invaded substrate by a

succession of heterotrophic micro-organisms, and Newman & Norman (1943) suggested that the nature of the available energy materials largely determined the fungal flora. Hawker (1950) considered that the formation of 'raw humus' on acid soils was due to the sensitiveness of bacteria to an acid medium, and to the relative inefficiency of fungi compared with bacteria as decomposers of cellulose. The acidity of the cores of tussocks of *C. paniculata* may therefore play a direct part in the formation of these tussocks, which consist largely of old leaf bases that have not been completely decomposed.

It is possible to segregate the fungal flora of *C. paniculata* into the initial colonists of moribund and newly dead leaves, and into the inhabitants of the residual products of decomposition. Such a division was suggested by Thom & Morrow (1937) for soil fungi, but, as they pointed out, the distinction between the two groups is to a certain extent theoretical. Members of the second group were able to compete satisfactorily with the initial colonists, and were frequently isolated from the outer leaves. However, the converse did not apply, and the initial colonists, such as the perithecial and pycnidial species, *Cephalosporium acremonium*, *Cladosporium herbarum* and *Fusarium culmorum*, were relatively infrequent in isolations from the interior of the tussock. *F. culmorum* was considered by Broadfoot (1934) to be a weak parasite on wheat. The numerous isolations of this species from the outer leaves of *C. paniculata* and its virtual absence from the interior of the tussock, together with its presence in the still living portions of leaves, would be consistent with such a weakly parasitic role.

Species of *Phoma* were isolated from surface-sterilized outer leaves on ninety-one occasions, but species of this genus were not collected as pycnidia in host tissues. Neither Grove (1935) nor Cunnell (1956) mention finding species of *Phoma* on *Carex*.

The main environmental factors which appeared to influence the seasonal activities of the fungi were temperature and water content of the substrate. There was no apparent correlation between rainfall and the isolation totals, while the effect of the hydrogen-ion concentrations may be linked with temperature, as the curves for these two factors are somewhat similar (Fig. 2). Figs. 2-4 show that fluctuations in total number of isolations in all leaf categories agreed in general with temperature during the summer months, but not during the winter. When water content of the outer and inner leaves was high, number of isolations tended to be low, regardless of the temperature. This can readily be seen for the October isolations from the outer leaves, and for the November isolations from the inner leaves. In November, the water content of the outer leaves had fallen, with a corresponding rise in number of isolations, despite a fall in temperature. There was a similar rise in isolations from the inner leaves in December, but this was accompanied by a slight rise in temperature. Species of *Penicillium*, particularly *P. spinulosum*, which provided the bulk of these isolations, would therefore appear to be adversely affected by a high-water content in the substrate, especially when the temperature was low. However, in the interior of the tussock, where the water content was constantly high, temperature had a direct effect on number of isolations of *P. corylophilum*, *P. notatum* and *Trichoderma viride*.

The rapid increase in number of isolations of *Fusarium culmorum* in July and August 1952, can be directly correlated with increases in temperature. Samuel & Greaney (1938) found a similar flare-up of *F. culmorum* on wheat plants in August and suggested that this invasion was a result of loss in vitality of the wheat after flowering. *Carex paniculata* flowers in May and June, and the fruit ripens in July; this cycle may be an additional factor influencing frequency of occurrence of *Fusarium culmorum*.

Bearing in mind the statement of Chesters (1949) that many fungi are introduced into the soil in organic matter from above the ground, it is interesting to find so many members of the so-called 'soil fungi' in this leaf litter, which would appear to be, first, a new substrate to be invaded by fungi growing on debris already in the soil, and, later, to be an important agent causing the continual addition of active fungal mycelium to the soil.

I am extremely grateful to Prof. C. G. C. Chesters for suggesting this topic, for his advice, and for the interest he has shown in this work.

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(Accepted for publication 31 July 1957)

Trans Brit. mycol. Soc. **41** (2), 196-202 (1958).

THE INVALIDITY OF THE GENUS *PYTHIOMORPHA*. II

By GRACE M. WATERHOUSE, M.Sc.

Commonwealth Mycological Institute, Kew

Of the five fungi that have been placed in *Pythiomorpha* Petersen, four are undoubtedly species of *Phytophthora* de Bary. *P. gonapodyides* (Petersen) Buisman must stand, as it was the first species to be described as having proliferating sporangia, a good specific character. *P. oryzae* (Ito & Nagai) Waterhouse comb. nov. appears to be a good species on account of its large sporangia associated with large oogonia and oospores. *Pythiomorpha miyabeana* Ito & Nagai I would place in synonymy with *Phytophthora megasperma* Drechsler. *Pythiomorpha undulata* (Petersen) Apinis is best retained as *Pythium undulatum* Petersen for the present. *Pythiomorpha fischeriana* Höhnk must remain a doubtful entity.

When Blackwell, Waterhouse & Thompson (1941) assembled evidence in support of the view that the genus *Pythiomorpha* Petersen should be included in *Phytophthora* de Bary, there remained the task of redispersing those species not already transferred. An attempt is now made to settle this problem and at the same time to discuss the position of the one species previously transferred.

PHYTOPHTHORA GONAPODYIDES (Petersen) Buisman = *Pythiomorpha gonapodyides* Petersen

The type of *Pythiomorpha*, *P. gonapodyides*, was described (Petersen, 1909) from mycelium and sporangia in two collections from natural substrata (submerged apples and alder twigs). The fungus was not isolated or brought into culture on artificial media. By courtesy of the Rijksmuseum, Copenhagen, I have been able to examine two slides which, according to their labels, were made (one apparently a day or two after the other) from this material by Petersen in 1904. They both bear the inscription 'Dam i Frederikshaabs Have, Glostrup.', one of the collecting grounds mentioned in the original account. The appearance of the fungus mounted unstained on these slides [there is no indication of how this was done], coupled with Petersen's description and figures, leaves no doubt in my mind that the species is rightly placed in the genus *Phytophthora*, even in the absence of oogonia and antheridia. Buisman (1927) declared this to be so and made the necessary transfer. She did not consider, however, the possibility that the species might be synonymous with one described earlier, and this point must be examined before it can be accepted.

Of the species of *Phytophthora* named before October 1909, none, as far as I have been able to discover (Waterhouse, 1956) is characterized by sporangiophores which renew growth within the emptied sporangium (internal proliferation), or which bear sporangia with very inconspicuous apical thickening (the latter feature is figured by Petersen, but not so

described by him, though it is apparent in his slides). These characters are undoubtedly specific, and it seems certain that *P. gonapodyides* is the earliest described species of *Phytophthora* possessing them. It should, therefore, stand as a good species, provided that one can be sure of identifying the fungus Petersen described. As his account is very brief and incomplete, a full description of the fungus, as far as could be ascertained from the two slides from the Rijksmuseum, Copenhagen, is given here.

Description

The young hyphae measure 3μ diam., mature hyphae average 8μ , sometimes reaching $9-12\mu$; they are very irregular in width with occasional inflations and narrow constrictions, but true hyphal swellings as defined by Blackwell (1949), are uncommon, and there are no groups of small angular swellings arranged in a net-like fashion as in *P. cryptogea*. Most of the hyphal branches arise strictly at right angles and are almost invariably narrowed at the base adjacent to the parent hypha. This construction is sometimes rendered more conspicuous by the widening of the branch beyond it, though normal width is soon resumed farther along. The hyphal wall is thickened at the constriction, sometimes only a little, sometimes heavily, even rarely to the occlusion of the lumen. There are very few transverse septa even in the apparently oldest hyphae; they are simple, transverse or diagonal, straight or curved, and occasionally slightly or deeply thickened at the centre. There are bright granules in the younger hyphae and hyaline aggregations at some of the constrictions.

The sporangia range from broadly to elongated obpyriform. The base is broadly flattened or, occasionally, tapered. The apex does not protrude as a papilla; it has retained its convex outline (not flattening as often happens), even though the preparations had dried out and had to be revived again; the hyaline apical thickening is extremely shallow. The opening left after dehiscence is medium broad, the sporangial wall then becomes crumpled, and the sporangiophore proliferates internally. Subsequent sporangia are either 'nested' within the earlier ones or occur at intervals along the sporangiophore, each one at some distance beyond the preceding. The sporangiophore is more slender than the other hyphae; the cross-wall delimiting the sporangium develops at a short distance below the basal plane and has no conspicuous plug. Measurements of three mature sporangia were $42 \times 20\mu$, $47 \times 30\mu$, and $70 \times 32\mu$ which indicate that Petersen's average ($44 \times 22\mu$) was somewhat low. There was no sign of repeated emergence of zoospores (empty cysts) among the mycelium and matrical debris on the slides.

Owing to our incomplete knowledge of *P. gonapodyides*, it is difficult to decide whether any fungi subsequently described under other names might be referred to this species. Most of the records of *P. gonapodyides* appearing in the literature subsequent to 1909 (Blackwell *et al.* 1941) are of no help in clarifying the position since most of them were mere notifications of the presence in gross cultures on natural substrata of a fungus having internally proliferating sporangia with dimensions

approximating to those given by Petersen. There is no certainty that the various collectors had all found the same species, or indeed that any of them had *P. gonapodyides*, since there are at least three species of *Phytophthora* with sporangia of this type, and the records might refer to any one of them.

Oogonia and antheridia in association with sporangia considered to conform to those of *P. gonapodyides* were described by von Minden (1916), Kanouse (1925), and Forbes (1935). Von Minden's record is a doubtful one, as he himself states, since the fungus was in mixed culture on the natural substratum and the immediate connexion of the oogonia with the mycelium bearing the sporangia (of which no measurements were given) could not be established. Moreover, the oogonia were in the empty hyphae of a species of *Saprolegnia* or *Achlya*, a most unusual habitat for a *Phytophthora*. It seems unlikely, therefore, that these oogonia were connected with the sporangia.

Kanouse reported oogonia in gross mixed cultures on agar and in single-spore (?zoospore) cultures on prune agar. It is not clear whether her descriptions and drawings were made from both these sources or from the latter only. The majority of the oogonia were described as being $22-36\mu$ diam. and without antheridia, a very exceptional state of affairs in *Phytophthora*; in fact, all the figures except one appear to be of chlamydospores, not oogonia. The antheridia described were found only in the single-spore cultures which were made directly from the original mixed culture. They were paragynous and wound round the oogonium, a feature not described elsewhere for *Phytophthora* though not unusual in *Pythium*. A culture at the Centraalbureau voor Schimmelcultures, Baarn, supplied by Kanouse under this name, does not conform to her published description (Blackwell *et al.* 1941).

Forbes used malt agar, and her general method (for a number of water moulds) was to isolate single hyphae bearing sporangia or oogonia. It is presumed that she used this method also for her *Pythiomorpha* isolates. She obtained dark brown to pale yellow oogonia ($26-35\mu$ diam., mean 30.5μ) with declinous paragynous antheridia ($11-15\mu$ wide); brown oospores (30μ) completely filled the oogonium. The brown colour might be attributed to the malt agar, as this coloration of normally hyaline or pale yellow oogonia of *Phytophthora* species commonly develops on this medium. Sporangia were not described or figured, presumably because they did not differ appreciably from those described by Petersen. I know of no species of *Phytophthora* which fits Forbes's description, though there is a small-spored form of *P. megasperma* (Tompkins, Tucker & Gardner, 1936) which comes near it. This form is considered again below.

As there is no certainty that these fungi represent Petersen's species it seems best to reject them, at present, as indeterminable and of no help in establishing *P. gonapodyides* on a firmer basis. The only way in which this can be done is by securing an isolate from the original habitat. Meanwhile there is no obstacle to the retention of the name *P. gonapodyides*.

PYTHIOMORPHA UNDULATA (Petersen) Apinis = *Pythium undulatum* Petersen

The second species to be included in *Pythiomorpha* was *P. undulata* (Apinis, 1930). The transfer of *Pythium undulatum* Petersen appears to be based on three collections made by Apinis in Finland, two from plant debris in water (both admittedly a mixture of water moulds) and one on submerged apples, all the material on which the study was made thus being in gross mixed cultures on natural substrata straight from the collecting grounds. *P. undulatum* is very common in fresh water on decaying plant remains, and there seems to be no doubt from his measurements of sporangia and illustrations that Apinis (1930; figs 2, 7) had this fungus. It is doubtful, however, whether the dehiscent sporangia containing encysted zoospores and the zoospore cysts showing repeated emergence, also depicted (figs. 3, 4 and 6), represent the same fungus.

No reasons for the transfer from *Pythium* were given. Though the coarse mycelium, large ovoid papillate sporangia (up to 167μ), and the numerous chlamydospores (sometimes called 'gemmae' or resting spores) of *P. undulatum* are not features one is accustomed to associate with *Pythium*, the frequent presence of a sporangial discharge tube and the delayed fashioning of the zoospores until the vesicle is forming are certainly diagnostic of the genus. And as the general consensus of opinion (Dissman, 1927; Drechsler, 1946; Goldie-Smith, 1952) is that the species is best left in *Pythium*, there the matter might be left until the sexual organs are found. The possibility that Apinis may have been dealing with two distinct fungi, a suggestion made previously by one or two workers, and that one fungus may have been a *Phytophthora* does not affect the issue, except in so far as to emphasize that his name change is not acceptable.

PYTHIOMORPHA MIYABEANA Ito & Nagai = *Phytophthora megasperma* Drechsler

In 1931 two species of *Pythiomorpha* were described from rice in Japan (Ito & Nagai, 1931), having been fully studied in artificial culture. *P. miyabeana* was stated to produce proliferating sporangia $36-53 \times 17-36$ (26.5×47) μ and dark yellow oogonia $28-50.5\mu$ diam., with oospores $24-36$ [probably a misprint for 46] μ and paragynous antheridia. A subculture, descended from the original isolate was obtained from Baarn, and it (I.M.I. 34386) appears to conform in most respects to the published description. The only differences I have been able to detect are that occasionally antheridia are amphigynous and the oogonia are only faintly yellow on most media.

This fungus appears to be almost identical morphologically with the one described (Tomkins *et al.* 1936) from a root rot of cauliflower in California and identified as *Phytophthora megasperma* Drechsler. Though the oogonia ($32-46\mu$ diam., av. 38.3μ) were consistently smaller than those of the type (given by Tomkins *et al.* as $36-52\mu$, av. 42.6μ , compared with the av. 47.4μ of Drechsler, 1931) they fell within the range of the original

diagnosis (16–61 μ). I have not seen a culture of the cauliflower fungus, but I have studied two isolates, one from soya-bean roots (I.M.I. 68258) in Ontario and the other (I.M.I. 68030) from peach roots in New South Wales, which appear to agree with it in all their morphological features. Miss Blackwell tells me, also, that the water mould found by Mrs Topping and identified by Mr S. F. Ashby as *P. megasperma*, which led to the first discussion of the invalidity of *Pythiomorpha* (Blackwell *et al.* 1941), was considered by him to be a small-spored form. I can see no reason for separating these fungi from *Phytophthora megasperma*, even though the maximum size of the oogonia and oospores is consistently and appreciably less than in the type.

PYTHIOMORPHA ORYZAE Ito & Nagai (1931) p. 51 = ***Phytophthora oryzae*** (Ito & Nagai) comb.nov.

The second species described by Ito & Nagai (1931) from rice was reported to have proliferating sporangia $41\text{--}84 \times 26\text{--}48 \mu$, mostly $60\text{--}84 \times 29\text{--}43 \mu$, and thus unusually large for a species of *Phytophthora*. A subculture of the original isolate, from Baarn (I.M.I. 45904), conforms to the original description. Species of *Phytophthora* with sporangia of comparable dimensions and form differ from the rice fungus in being specific to a certain host (not rice) or in producing oospores abundantly in culture or in the host, or in possessing some distinct morphological feature. The Japanese workers did not report oospores nor have I ever obtained them on a variety of media. When grown with *P. drechsleri* Tucker (I.M.I. 40499), however, the production of sexual organs was so abundant that the culture was tinged yellow. When measurements of the oogonia and the oospores were made, it was found that they fell into two groups, one within the range of *P. drechsleri*, with hyaline or pale yellow oogonia up to 45 or rarely 50 μ diam., and the other with amber-coloured oogonia often over 50 μ , sometimes up to 70 μ , and having a slightly thicker wall. Moreover, attached to some of the larger oogonia were paragynous antheridia, others having amphigynous antheridia, which are an invariable feature of *P. drechsleri*. It seems that the presence of the latter had induced the formation of sexual organs in the rice fungus. The strain of *P. drechsleri* used has occasionally produced sexual organs in monoculture on maize agar. In one such culture the oogonia were yellow and measured 36–56 (av. 48) μ diam. In dual culture with *P. drechsleri* I.M.I. 40532, the oogonia averaged 38 μ ; these, I suspect, were mostly of the latter strain because 40499 appears from experience to be an antheridial strain and 40532 an oogonial. A full description of the sexual organs of *P. oryzae* must of necessity await their appearance in monocultures, because it is not always possible to determine from dual cultures the source of the antheridium or indeed that of the oogonium. There is no doubt in my mind that *Pythiomorpha oryzae* should be placed in the genus *Phytophthora* and that it is sufficiently distinct from the species hitherto described, at least up to 1931, for it to be named as *P. oryzae* (Ito & Nagai) Waterhouse.

PYTHIOMORPHA FISCHERIANA Höhnk

The last species of *Pythiomorpha* to be described (Höhnk, 1936) was found on alder twigs in an unspecified locality (Wisconsin or Germany). It is difficult to understand why this fungus was ever put in the genus. Though the author stated that it 'agreed with the diagnosis and corresponded to the description as given for *Pythiomorpha*', this is not so, because Petersen's fungus was described as having 'sporangia proliferous several times', whereas Höhnk said that in his 'no proliferation was observed'. The sporangia described and figured by Höhnk are undoubtedly those of a *Phytophthora*. Oogonia 20–28 μ diam. were found 'after 3–4 months' [? in the original collection], but no antheridia were detected, nor was it demonstrated that the oogonia were connected with the mycelium bearing the sporangia. A request recently to Dr Höhnk for a culture brought the reply that he no longer has this species. Because of the inadequate description it is not possible to say with certainty which species of *Phytophthora* this fungus might represent, though one might hazard a guess that it might be *P. syringae* (Kleb.) Kleb. in view of the moniliod inflations and constrictions in the intramatrical hyphae, the cymose (non-proliferating) branching of the sporangiophore, and the dimensions of the sporangia and oogonia. It seems best, however, in view of the incomplete data, to leave the fungus as *incertae cedis* and dismiss the name *Pythiomorpha fischeriana*.

I should like to thank the Rijksmuseum, Copenhagen, for their courtesy in sending Petersen's original slides, and also Miss E. M. Blackwell for reading this sequel to her original paper and for her helpful criticisms.

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(Accepted for publication 14 August 1957)

A CORRECTION

I am grateful to correspondents who have pointed out an error in my Presidential Address to the Society, (*Trans. Brit. mycol. Soc.* **35**, 1-18, 1952).

On p. 10 it is stated that '...for spores or pollen liberated near the ground under normal conditions of turbulence, 99.9% of the spores will be deposited within 100 m. of the source...when spores are liberated under relatively calm conditions...perhaps only five spores in a million travel further than 100 m.'

Clearly the rate of deposition claimed here is too high and the passage should be corrected to read as follows: '...for spores or pollen liberated near the ground under normal conditions of turbulence, 90% of the spores will be deposited within 100 m. of the source...when spores are liberated under relatively calm conditions...perhaps only five spores in ten thousand travel further than 100 m.'

The error arose because I misread an entry in Table 21 of my earlier paper (these *Transactions*, **28**, 26-72, 1945) on which the statement was based.

P. H. GREGORY

THE CLAVARIACEAE OF THE MUSSOORIE HILLS (INDIA). IX

BY E. J. H. CORNER

Botany School, University of Cambridge

K. S. THIND AND SUKH DEV

Botany Department, Panjab University, Amritsar, India

(With Plate 8 and 2 Text-figures)

Three new species with one new variety are described: *Ramaria brevispora* sp.nov., *R. brevispora* var. *albida* var. nov., *Clavulina mussooriensis* sp.nov., *Clavaria indica* sp.nov. Type collections are deposited in the herbarium of the Panjab University. Duplicate material in alcohol-formalin is at the Botany School, Cambridge.

This paper continues the description of new species from the Mussoorie Hills (5000-7000 ft. altitude in the north-west Himalayas), as part of the study of the Fungal Flora of the region undertaken by Dr Thind and his students. Previous contributions (see References) described 38 known species, 8 new species and 8 new varieties. Descriptions have been made from fresh material, supplemented with dried material and that preserved in formalin-alcohol. The number given to each species is the serial number for this Clavarioid Flora. Corner's classification (1950) has been followed.

55. *Ramaria brevispora* sp.nov. (Pl. 8, fig. 1; Text-fig. 2a)

Receptacula ad 14×6.5 cm., terricola multiramosa, trunco nullo, flavidula apicibus intensioribus, carne alba immutabili, insipida. Sporae $5.5-7.5 \times 4-5.5 \mu$, ochraceae, subverruculosae, late ellipsoideae vel subglobosae, uniguttatae. Basidia $40-50 \times 6-8 \mu$; sterigmata 4. Hymenium vix incrassatum, receptaculi parti inferiori deficiens. Hyphae $3-10 \mu$ latae, tenuiter tunicatae, afibulatae, haud ordine secundo septatae.

Fructifications up to 14×6.5 cm., gregarious, solitary, erect, large, radial, *trunk absent*, but with a long white embedded stem-like base, branching from the ground-level, fleshy, *light yellow with deep yellow blunt tips*, drying brown to reddish brown; branches polychotomous below, dichotomous above, profuse, unequal, in alternating planes, compact; the lower branches -1 cm. wide, rugulose, pubescent, sterile (hairs $4-6 \mu$ wide, hypha-like, thin-walled, straight or flexuous); upper branches smooth, fertile; *fresh white, unchanging*; smell and taste inparticular.

On soil in oak forest (*Quercus incana* Roxb.): India (The Upper Park Road, Mussoorie, 21 Aug. 1955, No. 228).

Spores $5.5-7.5 \times 4-5.5 \mu$, light brown to ochraceous, verruculose without distinct warts, *broadly ellipsoid to subglobose* or obovoid, the wall -0.5μ thick, with one large gutta filling three-quarters of the spore, apiculus -0.8μ .

Basidia 40–50 × 6–8 μ ; sterigmata 4, 3–8 μ long. *Hymenium* absent from the lower third to three-quarters of the fruit-body, amphigenous on the upper branches. *Hyphae* 3–10 μ wide, monomitic, septate without clamps at short to long (160 μ) intervals, thin-walled, hyaline, sometimes constricted at the septa, not secondarily septate.

This species comes nearest to *Ramaria flaviceps* Corner, Thind & Anand (1956), but it has shorter spores (7–10.5 × 4–5.3 μ in *R. flaviceps*), for which reason the specific epithet is chosen.

56. ***Ramaria brevispora*** Corner, Thind & Dev var. ***albida*** var. nov. (Pl. 8, fig. 2)

Receptacula alutacea basim versus albida.

Fructifications up to 12.8 × 8.2 cm., tan white, the lower branches and buried base marble-white as the flesh, attached by slender rhizomorphs.

On humus in oak forest: India (Kempty Road, Mussoorie, 29 Aug. 1954, No. 229).

Spores 6.8–7.8 × 5–6 μ , pale brown, globose to subglobose or broadly ellipsoid, verruculose without distinct warts, with a large gutta, the apiculus 0.8 μ long. *Basidia* 6–9 μ wide; sterigmata (2–) 4, 5–8.5 μ long. *Hymenium* thickening to 140 μ , absent from the white base and lower parts of the branches, but with sterile hairs on these parts 80 × 1–3 μ , hyaline, thin-walled, branched, septate without clamps. *Hyphae* 3–12 μ wide, the cells 258 μ long, without clamps.

Except for the lack of yellow colour, this variety agrees in all respects with the typical state.

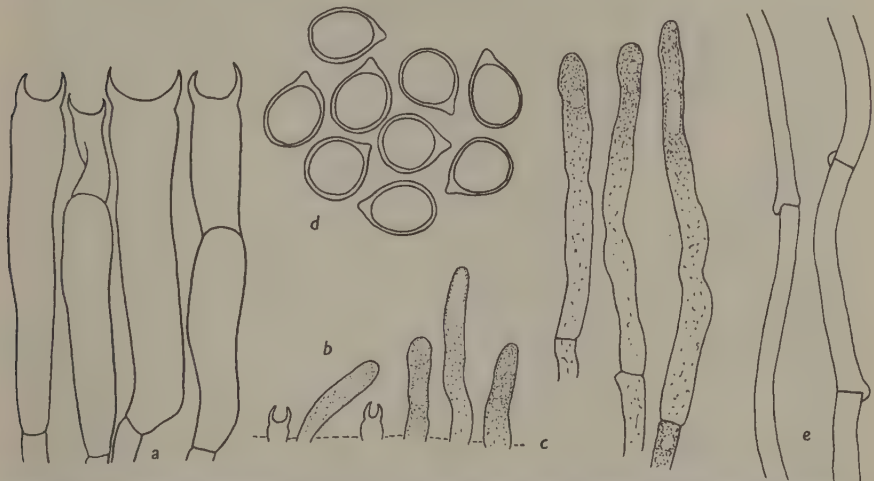
57. ***Clavulina mussooriensis*** sp. nov. (Pl. 8, fig. 3; Text-fig. 1, a–e)

Receptacula ad 8 × 4.5 cm., terricola, caespitosa vel gregaria, sparsim vel multiramosa, laevia vel tuberculis spiculisque aspera, albidocrenea dein fuscobrunnea vel brunneocinerea, apicibus albidis, ramis irregulariter polychotomis brevibus congestis cylindricis vel applanatis, aliquando palmatodivisis, insipida, carne brunneola. Sporae 7–9 × 5.5–8 μ , hyalinae, laeves, subglobosae vel obovoideae, uniguttatae. Basidia 45–60 × 5–7 μ , aetate ordine secundo septata, sterigmatibus 2 incurvatis. Hymenium incrassatum, hyphis solitariis sparsis hyalinis laevibus uniseptatis (sine fibula) 4–7 μ latis, 60 μ projicientibus transgressum. Hyphae 3.5–16 μ latae, hyalinae, tenuiter tunicatae, plus minus dense fibulato-septatae, cellulis usque 160 μ longis.

Fructifications up to 8 × 4.5 cm; gregarious, caespitose or solitary, medium-sized, erect, radial to flattened, trunk absent, sparsely to profusely branched, fleshy, smooth or roughened from tubercles and minute adventitious branches, cream-white becoming greyish brown or ash-brown with cream-white tips and immersed base, darker on drying; branches polychotomous, irregular, short, compact, radial to much flattened, longitudinally rugulose, smooth to rough, irregularly palmate in some young specimens, often fused, 5 mm. wide below, tips short and cristate or bifid, acute (sterile) or blunt (fertile); flesh light brown; smell and taste inparticular.

On soil in cedar forest (*Cedrus deodara*): India (Dhanolti, Mussoorie, 12 Sept. 1954, No. 230, *typus*); humicolous in cedar forest, Kanatal, Mussoorie, 22 Aug. 1956, No. 231, and Dhanolti, 23 Aug. 1956, No. 232.

Hymenium smooth, amphigenous, thickening to 200μ ; *emergent hyphae* sparse, solitary, $4-7\mu$ wide, hypha-like, obtuse, thin-walled, smooth, hyaline, straight or slightly bent, vacuolate, arising from the subhymenium and usually with a septum (without clamp) at the base, projecting $8-60\mu$. *Spores* $7-9 \times 5.6-8\mu$, hyaline, mostly obovoid, varying subglobose, smooth,



Text-fig. 1. *Clavulina mussooriensis* sp. nov., a, Basidia secondarily septate after spore discharge, $\times 1150$. b, emergent hyphae from the hymenium, $\times 500$; c, emergent hyphae drawn from their base, $\times 500$; d, uniguttate, smooth basidiospores, $\times 1150$; e, clamped hyphae, $\times 500$.

with a large gutta filling three quarters of the spores, apiculus -0.4μ long. *Basidia* $45-60 \times 5-7\mu$, subclavate, secondarily septate after spore-discharge; sterigmata, 2, incurved, $4-5.6\mu$ long. *Hyphae* $3.5-16\mu$ wide, hyaline, thin-walled, septate with clamps at short to long intervals (-150μ).

This resembles *C. cinerea* (Fr.) Schroet., but differs in the brown colour and in the emergent hyphae. These are too unspecialized to be regarded as cystidia, such as those of *C. leveillei* (Sacc.) v. Ov., but they are a normal feature of the fresh hymenium and not the effect of collecting. *C. hispidulosa* Corner, Thind & Anand (1956) differs in the grey colour of the usually simple fruitbodies and in the fasciculate emergent hyphae.

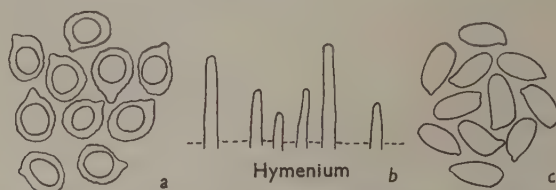
58. *Clavaria indica* sp. nov. (Pl. 8, fig. 4; Text-fig. 2 b, c)

Receptacula $15-40 \times 0.8-3$ mm., simplicia, obtusa, caespitosa, gregaria vel solitaria, glabra, laevia, fuligine ovel atro-umbrina, trunco sterili brunneo, carne subconcolori immutabili, insipida. Sporae $5.5-7.5 \times 2.5-3.2\mu$, subhyalinae, ellipsoideae, laeves, aguttatae, apiculo minuto. Basidia $25-40 \times 5.5-8\mu$, afibulata; sterigmata 4, ad 6μ longa. Cystidia $1.5-3\mu$ lata, ad 14μ projicientia, subcylindrica, sparsa, tenuiter tunicata, laevia. Hymenium ad 120μ vix incrassatum. Hyphae $2-13\mu$ latae, cellulis ad 270μ longis, tenuiter tunicatae, septatae haud ordine secundo, afibulatae, saepe ad septum constrictae.

Fructifications $15-40 \times 0.8-3$ mm., simple, caespitose in small clusters to 1 cm. wide, gregarious, or solitary, erect, fleshy, brittle, smooth, glabrous, sooty or smoky brown; trunk $1-6 \times 0.8-2$ mm., brown, lighter coloured than the club, sterile; club subfusiform or subcylindric, varying flattened and

sulcate, solid, blunt, fertile to the apex; flesh light brown, unchanging; smell and taste inparticular.

On humus in oak forest: India (The Park, Mussoorie, 26 Aug. 1954, No. 233).



Text-fig. 2. *Ramaria brevispora* sp.nov., *a*, verruculose, uniguttate basidiospores, $\times 1150$. *Clavaria indica* sp.nov., *b*, very narrow cystidia, $\times 1150$; *c*, aguttate, smooth, ellipsoid basidiospores, $\times 1150$.

Hymenium slightly thickening to 120μ . *Spores* $5.5-7.5 \times 2.5-3.2\mu$, subhyaline, smooth, ellipsoid, aguttate, minutely apiculate. *Basidia* $25-40 \times 5.5-8\mu$, elongate clavate, without a clamp at the base; sterigmata 4, straight or slightly curved, to 6μ long. *Cystidia* -3μ wide, projecting -14μ , thin-walled, subcylindric, smooth, sparse, straight or slightly flexuous. Hyphae $2-13\mu$ wide, cells to 270μ long, hyaline, thin-walled, septate at short to long intervals, not secondarily septate, without clamps.

The fuliginous colour, the hypha-like cystidia, and the caespitose habit in oak woods characterize this species. It comes near to *C. purpurea* Fr., which has much wider and copiously septate hyphae, and large cystidia, and to the little-known *C. nebulosoides* Kauffm. with paler brown colour: both these apparently occur only in coniferous forests.

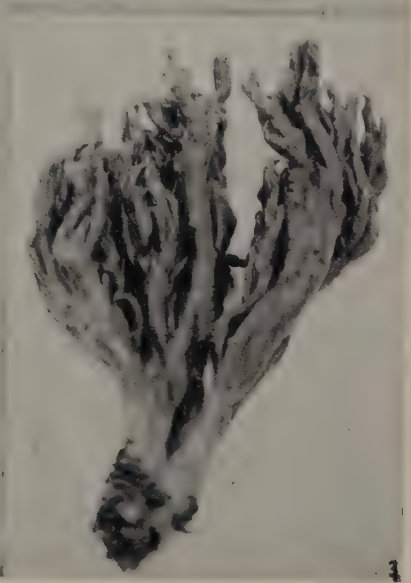
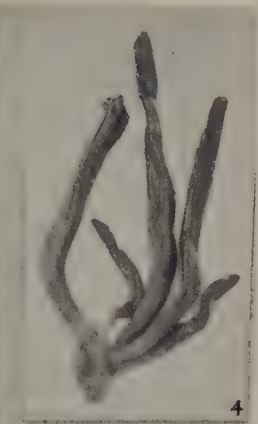
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EXPLANATION OF PLATE 8

- Fig. 1. *Ramaria brevispora* sp.nov. (No. 228).
 Fig. 2. *R. brevispora* Corner, Thind & Dev var. *albida* var.nov. (No. 229).
 Fig. 3. *Clavulina mussooriensis* sp.nov. (No. 230).
 Fig. 4. *Clavaria indica* sp.nov. (No. 233).

(Accepted for publication 14 August 1957)



OCURRENCE AND ESTABLISHMENT OF *PYTHIUM* IN SOILS

By R. BARTON

Department of Cryptogamic Botany, University of Manchester

(With 4 Text-figures)

Species of *Pythium* were isolated from cultivated soils by using susceptible host seedlings as baits, but could not be found in soils taken from woodland or other non-cultivated areas. No isolates were obtained from very acid soils in which *Trichoderma viride* was particularly abundant.

The behaviour of inocula of *Pythium mamillatum* in a cultivated (native) soil, and in a woodland (alien) soil was assessed by means of the host test described. Maize meal added to the cultivated soil became colonized by *P. mamillatum* after only 2 days, indicating, that under these conditions, the parasite behaves as a saprophytic 'sugar fungus'. In the alien woodland soil *P. mamillatum* did not colonize maize meal. After neutralization of this acid soil with lime, however, the fungus rapidly utilized the material. The parasite could not be grown in culture at the low pH value of the alien soil.

The behaviour of vegetative hyphae of *P. mamillatum* in these contrasting soils was studied by the glass-fibre tape technique. In native, cultivated soil, oospores and resting sporangia were formed abundantly, whereas in alien woodland soil these bodies were not produced. Thus the inability of *Pythium* hyphae to produce a resting phase may prevent long-term survival of the parasite in alien soils. This effect was shown to be due to low pH value of the soil, which may directly limit the normal life cycle of *Pythium* in acid woodland soils.

INTRODUCTION

Since the establishment of the now classical baiting technique for the isolation of *Pythium* spp. from soil (Butler, 1907), it has become recognized that members of this genus are undoubtedly true soil inhabitants, persisting there in a saprophytic as well as a parasitic capacity (Garrett, 1956). Despite this recognition, however, our knowledge of the group in this natural habitat lags far behind our present understanding of the parasitic behaviour of these fungi. Accordingly, a study of *Pythium* in soils from a contemporary ecological standpoint seems fully justified, this paper being an introduction to a more complete autecological investigation of the genus.

Although there is little doubt of the widespread occurrence of *Pythium* spp. in soils, some evidence suggests that in non-cultivated areas, these fungi are absent, or, at the most, sparsely distributed. Remy (1949) concluded that *Pythium* spp. were infrequent in forest soils and those of a dry, sandy nature, but were abundant in soils with animal or human contacts, i.e. cultivated soils. Similarly, Warcup (1952) showed that these parasites predominated in nursery soils, but were absent from soils taken in virgin forest areas. The question has been reconsidered in this investiga-

tion, in which the occurrence of *Pythium* spp. in a number of contrasting soils, and the factors affecting the ability of the fungi to become established in some soils and not in others, have been studied in some detail.

MATERIALS AND METHODS

Soils. Twenty-five soil samples from different parts of the British Isles were tested for the presence or absence of *Pythium*, for occurrence of other soil fungi, and for pH value and organic content. Samples were taken from the upper 6 in. of the soil profile; for woodland soils the surface litter was removed prior to collection. Each soil sample was stored in a sealed Polythene bag (14 × 8 in.). Determinations of pH were made with a Cambridge glass-electrode meter, following the method given by Piper (1950), using a 5 water:1 soil ratio. The percentage of organic material in each soil was determined by ignition. Soil plates (Warcup, 1950) were used to ascertain the principal soil fungi.

Two of the soils were selected for further experimental studies. Soil A (soil 4 in Table 1), chosen as a representative example of cultivated soil, was obtained from a garden plot in south-west Lancashire. The soil was sandy with a moderately high organic content. For several years the garden had been devoted to the cultivation of potatoes, occasionally alternating with crops of peas and beans: farmyard manure had been added annually. The pH value was 5.4, organic content 13.7 %, and the moisture-holding capacity (M.H.C.), as determined by the method of Keen & Raczkowski (1921), was 48 g./100 g. air-dry soil.

Soil B (soil 1 in Table 1), taken as a representative of woodland soils, was from the floor of a deciduous forest. It supported a ground flora of *Festuca*, *Poa* and *Endymion* beneath a stand of *Quercus* and *Acer*. The surface litter of dead leaves had accumulated above a rich dark organic soil, below which lay a subsoil of sand. The pH value of this soil was 3.7, organic content 16.3 and M.H.C. was 72.

Isolation of Pythium. Standard isolation techniques were tested to find the most suitable one for isolating *Pythium* from soils. The one finally adopted involved the use of living seedlings as soil 'baits'. Turnip seedlings (cultivar Clibran's White Ball) were selected for this purpose, as combining a high degree of susceptibility to *Pythium* with ease of handling. The test soil, in 2½ in. plant pots, was moistened with tap water and sown with twenty-five turnip seeds. Pots were incubated in a germinator receiving natural light, and maintaining a temperature not less than 25° C. and a relative humidity of 80–100 %. After 6 days, the seedlings had grown to an average height of 2 in.; if *Pythium* was present, many exhibited damping-off symptoms. Diseased seedlings were washed in 0.1 % mercuric chloride solution for 1 min., rinsed in sterile water, and plated on oat agar. *Pythium* colonies developed rapidly from any diseased parts. The fungus was freed from the bacteria, nematodes and amoebae continually found associated with fresh isolates; the most successful technique for this purpose was that devised by Sleeth (1945). For morphological observations, species of *Pythium* were grown on shallow plates of clear oat agar. Direct microscopical observation of these plates was made after flooding with

water to which one or two drops of phenolic Rose Bengal stain (Conn, 1932) had been added.

A seedling test for comparing the activity of *Pythium* in native and alien soils. McLaughlin (1947) and H. C. Smith (cited by Garrett, 1956) have used seedling tests to ascertain the activity of *Pythium* in soil, a method which implies that the number of seedlings showing damping-off symptoms is in fact a satisfactory indication of that activity. In practice, however, this method has been found to give highly variable results and suffers from several disadvantages. First, on becoming established on one seedling, *Pythium* grows outwards over the remainder in a very short time, so that the number of seedlings attacked does not reflect the initial abundance of the parasite in the soil. In addition, the resulting tangled mass of seedlings makes correct counting extremely difficult. Secondly, Leach (1947) has shown that environmental factors may markedly affect this disease through host resistance, which is influenced by external conditions. The number of damped-off seedlings obtained in this method, then, may depend more upon variation in host resistance than upon the activity of *Pythium* under different soil conditions. Thirdly, damping-off symptoms may be caused by fungal parasites other than *Pythium*, or may even be due to faulty growing conditions (Beach, 1946). For instance, in the woodland soil investigated here, seedlings developed slowly and the roots often rotted away even in the absence of *Pythium*. It was thus impossible to compare woodland soil with a cultivated soil, in respect of the activity of *Pythium* inocula, by this method thus used.

To offset these difficulties, a satisfactory technique requires that healthy seedlings be left in contact with soil for the minimum period, after which determinations of the presence or absence of *Pythium* in all diseased plants are necessary. A modified baiting technique has therefore been devised whereby germinated seeds are placed in soil for 48 hr. only, then surface sterilized and plated on agar to record the number attacked by the fungus. By this technique, turnip seeds were germinated on moist filter-paper in Petri dishes at a temperature of 25° C. for a period of 24 hr.; twenty germinated seeds were placed upon 20 g. test soil in a 100 ml. conical flask. The flasks were plugged with cotton-wool to reduce moisture loss, and incubated for 2 days under artificial light (16 hr./day) at 22° C. The seedlings were then washed thoroughly in water, and wrapped in a small square of muslin before passage through 0.1 % mercuric chloride for 1 min., and three changes of sterile water. Finally the seedlings were plated on oat agar, and incubated for 2 days at 20° C. At the end of this period *Pythium* colonies could be identified, and the percentage of infected seedlings was recorded.

The method has two main disadvantages. First, the seedlings are in contact with soil for 48 hr. only, so that the presence of *Pythium* in the active phase only would be recorded. Secondly, a certain proportion of the seedlings are naturally resistant to the attacks of the parasite; for this reason, the same strain of seeds was used throughout the experimental work.

Tests carried out on soil A, from which *P. mamillatum* was originally isolated (see Table 1), indicated that the natural inoculum could not be

detected by the short baiting period in this technique, and could reasonably be ignored when planning subsequent experiments.

The glass-fibre tape technique for the direct observation of Pythium hyphae in soils. Several methods have been devised for direct observation of fungi in soil. Legge (1952) suggested that glass-fibre tapes and threads could be used as inert bases upon which fungal development in soil could be examined microscopically. This technique, originally used for the study of fungal spores, has been adopted here for study of *Pythium* hyphae in soil.

Glass-fibre tape, 1.5 cm. wide, was divided down the middle and cut into sections *c.* 5 cm. in length. The tapes thus prepared were soaked in 70 % alcohol for 1 hr. and were then autoclaved for 1 hr. at 15 lb./sq.in. pressure in Petri dishes. The sterilized tapes were laid flat on the surface of malt agar plates, which were then inoculated centrally with *P. mamillatum*. Most species of *Pythium*, when grown on malt agar, tend to produce a thick mycelial mat, and the development of oospores and resting sporangia is delayed for some 5-6 days. This is so with *P. mamillatum*, and it is therefore possible to remove the tapes before oospores have developed. Before removal of the tapes after 2 days at 25° C., the plates were surveyed at a low magnification for evidence of bacterial contamination and oospore formation. For microscopical examination after a period in soil, the tapes were stained in steaming phenolic Rose Bengal for 1 min. (Conn, 1932), then washed and mounted in water.

Selection of the test fungus. *Pythium mamillatum* Meurs was used as the test fungus throughout this investigation. This species, which was originally isolated from the Lancashire garden soil, offered the advantage of a spiny oogonium wall clearly characterizing the oospores, so that there was little danger of confusing this stage with resting sporangia or chlamydospores, both of which have smooth exteriors. The distinction between these two stages in species of *Pythium* is often difficult to make in culture, and Warcup (1952) also has encountered some difficulty in deciding the exact nature of *Pythium* structures isolated from soil. The morphology of *P. mamillatum* is illustrated in Fig. 1.

FACTORS AFFECTING THE OCCURRENCE OF *PYTHIUM* IN SOILS

Ten out of twenty-five soils investigated were shown to contain *Pythium*. These soils were obtained from cultivated areas, the remaining fifteen being from natural, non-cultivated localities. These observations, together with the pH value, organic content, texture, and predominant members of the fungal flora of each soil are presented in Table 1.

From this information it is possible to infer that *Pythium* is present in cultivated soils but not in those from 'natural' areas. So much evidence has accumulated from reports of the pathogenicity of *Pythium* spp. on cultivated crops that there seems little doubt of their general occurrence in cultivated soils. Indeed, as Chesters (1949) has already pointed out, *Pythium* is specially prolific in cultivated soils under vegetable crops. The sparseness or absence of this fungus in woodland soils has been indicated by Remy (1949) and Warcup (1952). A cultivated and a 'natural' soil differ in several ways; for instance, cultivation practices alter the

pH value, the physical structure, the type of organic material, and the nutrient status of a soil. Cultivated soils are also characterized by a predominance of short-life-cycle weeds, the annual or ephemeral habit of which provides a continuous supply of organic matter for fungi such as

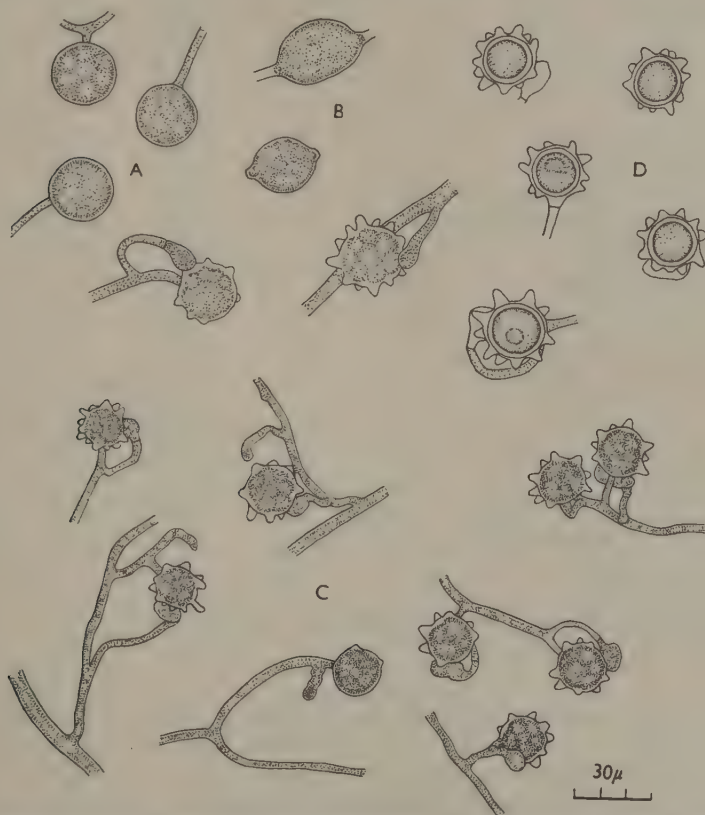


Fig. 1. Stages in the life cycle of *Pythium mamillatum*. A, spherical sporangia; B, intercalary chlamydospores; C, oogonia and antheridia, showing typical protuberances on the oogonial wall, and monoclinal antheridia; D, mature oospores.

Pythium. An experimental investigation of these and other factors in relation to *Pythium* is therefore desirable. However, some insight into the problem can be obtained by correlating the distribution of the fungus in soils with certain of their physical characteristics.

Soil pH value. Of the soils investigated, *Pythium* occurred only in those within the pH range 5.5-7.5. Warcup (1952) found *P. ultimum* abundant in nursery soils with pH 6.8-7.2, and rare in soils with pH 5.3-5.5. Remy (1949) recorded *Pythium* in soils with pH values above 4.5. Roth & Riker (1943) have pointed out that the pathogenicity of the fungus is greatest between pH 5.5 and 8.0, while both Buchholtz (1938) and Takahashi (1952) regard the optimum pH for growth of *Pythium* spp. as 6-7. The presence of the parasite in soils is thus correlated with slightly acid or neutral

Table 1. Occurrence of *Pythium* species in a number of soils

No.	Location	<i>Pythium</i> spp.	Abundance of		pH	% organic content
			<i>Trichoderma</i> <i>viride</i>	Texture		
1	Deciduous wood (Lancs)	.	+++	Sand	3.7	16.3
2	Coniferous wood (Lancs)	.	+++	Sand	3.3	65.5
3	Heath (Lancs)	.	+++	Shale	3.7	30.9
4	Veg. garden (Lancs)	<i>P.m.</i>	+	Sand	5.4	13.7
5	Flower garden (Lancs)	<i>P.d.</i>	+	Clay	6.4	9.7
6	Beech wood (Ches.)	.	+++	Sand	3.8	6.8
7	Greenhouse (Lancs.)	<i>P.d.</i>	+	Loam	6.4	15.3
8	Old pasture (Ches.)	.	++	Sand	4.6	11.6
9	Hedgerow (Ches.)	.	++	Sand	4.3	16.5
10	'Oat' field (Monmouthshire)	<i>P.?</i>	+	Clay	5.9	6.9
11	Birch wood (Monmouthshire)	.	+++	Clay	4.3	11.8
12	Veg. garden (Devon)	<i>P.u.</i>	—	Stony	7.7	11.5
13	Hen-run (Lancs)	.	++	Clay	4.5	11.7
14	Waste land (Lancs)	.	++	Sand	4.2	4.7
15	'Potato' field (Lancs)	<i>P.d.</i>	+	Clay	5.9	4.6
16	'Wheat' field (Lancs)	<i>P.u.</i>	+	Loam	6.0	5.6
17	'Oat' field, hill-land (Lancs)	<i>P.m.</i>	+	Clay	5.8	6.0
18	Flower bed (Southport)	<i>P.u.</i>	+	Loam	7.4	3.6
19	Sphagnum bog (Ches.)	.	+++	Black and sticky	3.7	11.0
20	Sheep pasture (Ches.)	.	++	Stony	4.1	11.0
21	Sheep pasture, limestone (Ches.)	.	++	Stony	5.3	12.0
22	Sand dune (Southport)	.	+	Sand	7.4	1.0
23	Deciduous wood (Ches.)	.	+++	Sand	4.3	14.0
24	Flower bed (Bournemouth)	<i>P.u.</i>	+	Stony	7.6	6.3
25	Coniferous grove (Bournemouth)	.	+++	Stony	5.2	26.0

P.m. = *Pythium mamillatum*. *P.d.* = *P. debaryanum*. *P.u.* = *P. ultimum*. +++ = dominant; ++ = abundant; + = present; — = absent.

conditions. It remains to relate this evidence to the fact that *Pythium* occurs in cultivated but not in 'natural' soils. Soils under crops generally have a pH range of 5.5–7.5, i.e. the optimum range for *Pythium* spp. 'Natural' soils afford a wider range of pH values. In the series examined by the writer, the few natural soils with pH > 5.5 did not yield *Pythium*, but Remy (1949) found it in woodland soils with pH values of 6.0–6.8, and Stenton (1953) isolated it from fen soil with pH 7.0. Thus in natural soils with pH values below 5.5 the absence of *Pythium* can be correlated with the acid reaction. In natural soils with pH values above 5.5, where the reaction is not limiting, the absence of *Pythium* must be ascribed to some other factor. The few reports of the fungus in these soils suggest that this factor may not be an absolute one.

Soil texture. The soils examined ranged from almost pure sand to clay. There was no correlation between the presence of *Pythium* and soil texture, since both sandy and clay soils which had been cultivated harboured the pathogen, whereas woodland soils of either nature were free. On the other hand, Remy (1949) found that clay soils contained more of this parasite than did sandy soils, and Vámos (1954) showed that damping-off of pine seedlings was more prevalent in clay soils. The activity of *Pythium* is presumably favoured in such soils by their increased water-holding

capacity and resistance to desiccation, but, as pointed out above, this fact is unlikely to be an overriding influence in the distribution of *Pythium* in soils.

Associated fungi. In preparing fungal lists of the soils investigated, it was evident that in 'natural' soils, particularly those from acid woodland areas, *Trichoderma viride* was the dominant fungus, whereas cultivated soils supported a greater number of genera, *T. viride* being relegated to a less dominant position. The inhibition of plant pathogenic fungi in soil by *T. viride* has been demonstrated by Rishbeth (1951) for *Fomes annosus*, and by Bliss (1951) for *Armillaria mellea*. Weindling (1934) and Allen & Haenseler (1935) showed that *T. viride* was antagonistic to *Pythium* in culture, and it may well be that a similar situation exists in soils. Certainly *Pythium* was never isolated by the writer from any soil in which *Trichoderma viride* was abundant. At this stage, however, it is difficult to distinguish between the effects of low pH and the antagonistic action of fungi such as *T. viride*, which are closely interrelated.

INDIRECT STUDIES OF *PYTHIUM* INOCULA IN NATIVE AND ALIEN SOIL

Experiment 1. The effect of maize meal amendments on inocula in soils A and B. Since *Pythium* has been previously recorded as a soil saprophyte by de Bary (1887), Butler (1907) and many subsequent workers, its ability to colonize maize meal in soil was investigated. At the same time, some indication of the difference in the activity of the fungus towards this material in soil A (native garden soil) and soil B (alien woodland soil) was sought.

Soils A and B were allowed to become air-dry and were then made up to 50 % M.H.C. with tap water. Two samples of each soil were placed in large Meilleur jars, and 15 % by weight of *P. mamillatum* inoculum added. The inoculum was prepared by growing the fungus for 2 weeks on a sterilized mixture of moist sand and 3 % maize meal. Two per cent by weight of fresh maize meal was then mixed with one sample of each soil. There were thus four treatments, viz. soil A control, soil A + maize meal, soil B control, and soil B + maize meal. During the experiment the water loss from each jar was made up periodically. The activity of *P. mamillatum* under these different conditions was determined after 0(-2), 4(-6), 7(-9), 14(-16) days and then after 3, 5 and 12 weeks. For each sampling, three replicates of 20 g. soil were weighed out from each jar into 100 ml. conical flasks and the activity of the pathogen in these samples determined as already outlined. Counts of bacteria and fungi in the four treatments were made at weekly intervals by the dilution plate method. For fungi a dilution ratio of 1:5000 was suitable, and a ratio of 1:200,000 was used for bacterial counts. The results obtained are presented in Figs. 2 and 3.

In both unamended soils, there was a gradual decrease in the activity of the added inoculum, which fell after 5 weeks to a low level that could not be detected by the method in use. In soil A (the soil native for the test fungus), addition of maize meal caused a marked increase in activity of *P. mamillatum* during the first 2 days. This initial flush of activity can be ascribed to colonization of the fresh maize meal by the fungus, indicating then, that *P. mamillatum* is an active saprophyte in this habitat. In alien

soil B, no initial stimulation of activity by the addition of maize meal was obtained, the inoculum level gradually decreasing with time as in the unamended soil; the situation was thus in strict contrast to that found in

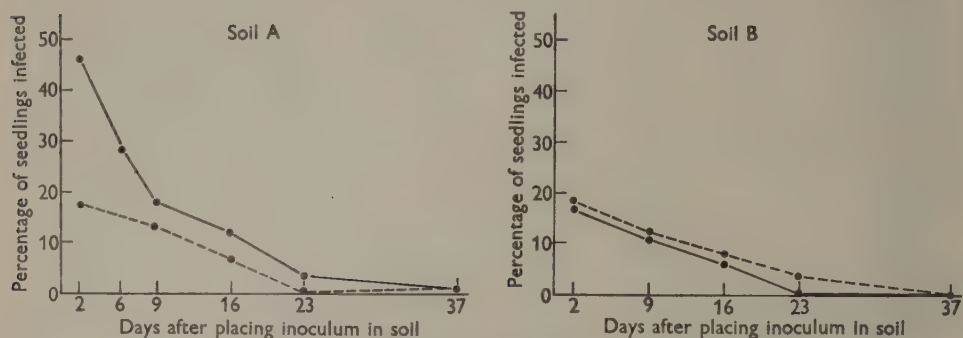


Fig. 2. Behaviour of inocula of *Pythium mamillatum* in soils A and B in the presence of maize meal. —●—, maize meal added; ---●---, unamended soil.

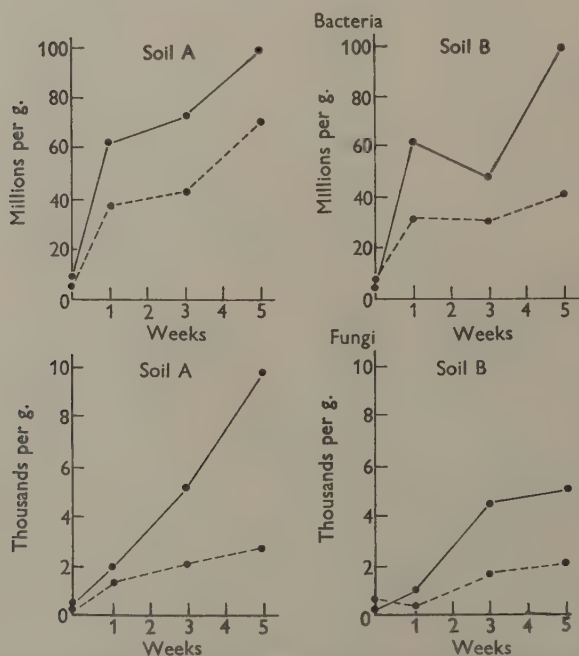


Fig. 3. Variation in numbers of bacteria and fungi in soils A and B amended with maize meal. —●—, maize meal added; ---●---, unamended soil.

native soil. Counts of indigenous soil organisms taken concurrently in these treatments show a significant increase in the flora due to the maize meal. This increase was, however, fairly gradual, so that in soil A, *P. mamillatum* had colonized the maize meal before the material could be utilized by the indigenous flora.

The inability of *P. mamillatum* to assume a saprophytic status in the alien soil is not altogether unexpected, since Kubiena & Renn (1935) pointed out that each soil determines its own microflora, and several investigators have been unable to establish fungi in soil alien to them. In the present instance, it appears that in the acid woodland soil, there is some factor or factors inhibiting the growth and activity of the added inoculum. This inhibition is sufficient to confer a decided advantage on the indigenous saprophytic fungi in this soil during the colonization of fresh organic substrates.

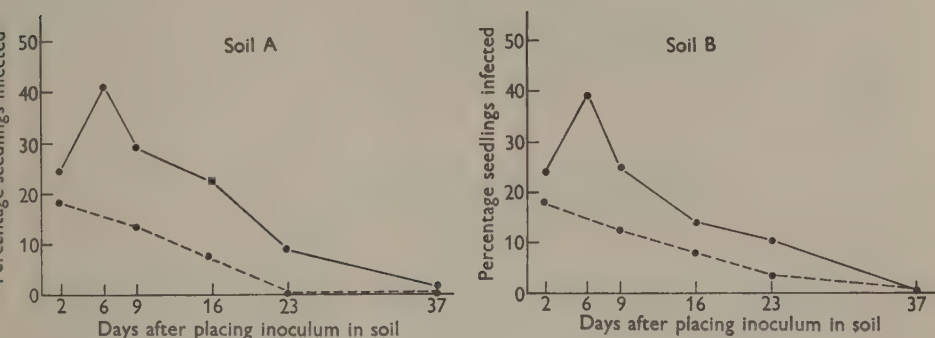


Fig. 4. Behaviour of inocula of *Pythium mamillatum* in soils A and B in the presence of turnip seedlings. ●—●, seedlings present; ●---●, seedlings absent.

Experiment 2. Effect of seedlings on inocula in soils A and B. During preliminary experiments, a considerable number of indigenous seedlings was observed to grow from soil A, but not from soil B. In view of this difference, it seemed desirable to investigate the effect of the growth of seedlings on the establishment of *P. mamillatum* in these soils.

Soils A and B which, after air-drying, had been made up to 50% M.H.C., were mixed with 15% of *P. mamillatum* inoculum and packed into 2½ in. plant pots. Six pots of each soil were prepared; three of each batch were sown with turnip seeds which were allowed to grow and develop in a normal manner; the remaining pots were left as controls. The activity of *Pythium* in the pots was estimated at intervals by placing twenty germinated turnip seeds between the growing seedlings, leaving them for 2 days, then surface sterilizing and plating-out in the manner described above. Tests were carried out at 0(-2), 4(-6), 7(-9), 14(-16), 21(-23) and 35(-37) days. The pots of soil were kept under bell-jars for this experiment and maintained in a moist condition by rolls of wet filter-paper. The results are presented in Fig. 4.

The behaviour of *P. mamillatum* in the presence of susceptible seedlings was similar in the two soils. There was a rapid initial increase in activity of the pathogen after 4-6 days. This fell off gradually, until, after 5 weeks, the original inoculum could no longer be detected by the method employed. The initial peak of activity coincided with the period when the seedlings were showing visible signs of *Pythium* attack. It appears then, that in both native and alien soils, the parasite, if present at a high enough

inoculum level, is capable of attacking host plants and utilizing the food base thus gained. The presence of host plants thus seems to be an important factor in the establishment of inocula of *Pythium* in soils.

Experiment 3. Effect of different soil moisture contents on inocula in soils A and B. Increased activity of *Pythium* under conditions of high-water content has been reported by Flor (1930), Roth & Riker (1943), Beach (1949) and Takahashi (1952). It was thus considered important to test the effect of this variable on the establishment of the fungus in native and alien soils. Both soils were air-dried and portions made up to 20, 50 and 80 % M.H.C. by adding tap water. Each treated soil was mixed with 15 % by weight of inoculum of *P. mamillatum*, and stored in Meilleur jars so that the water content could be maintained at a constant level. Tests for the activity of *Pythium* were made at 0(-2), 4(-6), 7(-9), 14(-16) and 28(-30) days (Table 2).

Table 2. *Percentage infected turnip seedlings from Pythium mamillatum inocula in soil A at different moisture contents*

	Replicates	Days in soil				
		0-2	4-6	7-9	14-16	28-30
20 % M.H.C.	1	0	0	0	0	0
	2	0	0	0	0	0
	3	10	0	0	0	0
50 % M.H.C.	1	0	5	10	0	0
	2	0	5	0	0	0
	3	10	5	10	0	0
80 % M.H.C.	1	25	5	9	15	0
	2	33	10	28	15	5
	3	0	15	22	15	0

P. mamillatum did not become established in soil B at any of the moisture contents examined, and so no data for this soil are given in Table 2. In soil A, results show that the higher the soil moisture content, the greater was the activity of *P. mamillatum*. These findings confirm those of McLaughlin (1947), who showed that desiccation of soils in summer decreased the frequency of *Pythium* on isolation plates, and also those of Remy (1949), who observed a greater quantity of this fungus in wet soils. The factor preventing the establishment of the pathogen in soil B was, however, unaltered by varying the water content.

Experiment 4. Effect on inocula of neutralizing soil B. It has been suggested earlier that soil acidity might prevent establishment of *Pythium* in alien soils. Therefore the reaction of soil B was altered from the original pH value of 3.7 to pH 7.1, by addition of calcium hydroxide, followed by an incubation period of 5 months; the behaviour of *P. mamillatum* in this soil was then compared with that in untreated soil B. In order to facilitate a closer comparison between the activity of the test fungus in neutralized soil B, and that in native soil A, a treatment incorporating maize meal with neutralized soil B was included in the experiment. Three treatments were thus prepared, normal soil B, neutralized soil B, and the latter with 2 % by weight of maize meal. These soils were maintained at 50 % M.H.C. and

15 % of *P. mamillatum* inoculum was added. Storage was again in Meilleur jars. Tests for the activity of *P. mamillatum* were carried out at 0(-2), 4(-6), 7(-9) and 14(-16) days (Table 3).

Table 3. *Percentage infected turnip seedlings from Pythium mamillatum inocula in untreated and neutralized soil B*

	Replicates	Days in soil			
		0-2	4-6	7-9	14-16
Untreated soil B	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
Neutralized soil B	1	10	0	5	0
	2	0	5	5	0
	3	10	0	0	0
Neutralized soil B + 2 % maize meal	1	25	5	10	15
	2	23	10	10	15
	3	17	0	10	15

In untreated soil B, *Pythium* activity was not detected at any time. In neutralized soil B, activity was relatively low. In neutralized soil, to which maize meal had been added, the activity of *P. mamillatum* increased considerably over the first few days and then fell off with time. Thus in neutralized soil B, *P. mamillatum* was able to become saprophytically active and utilize maize meal, which had previously been observed only in cultivated soil. From this experiment, it appears that the acid reaction prevents the establishment of inocula of *P. mamillatum* in the alien woodland soil. It is possible, however, that this is not a direct action of the acid conditions on the fungus itself. Reaction is known to influence many other related characteristics of soil, amongst which, a quantitative or qualitative change in the microflora would be especially relevant to this problem.

Experiment 5. Influence of pH of culture medium on growth of P. mamillatum. This experiment was undertaken to discover how far pH of the medium influenced growth of *P. mamillatum*, with a view to deciding whether the acid reaction of soil B was having a direct or an indirect action on the fungus. Sterilized 3 % malt solution was adjusted by addition of sterilized N/10 sulphuric acid or N/10 sodium hydroxide to pH values of 3, 4, 5, 6 and 7, and 20 ml. medium was placed in triplicate 100 ml. flasks for each pH. The flasks were then inoculated with 4 mm. disks from the edge of an agar colony of *P. mamillatum*. After 7 days, the mycelial mats were harvested, dried at 100° C., and weighed (Table 4).

Table 4. *Mycelial growth of Pythium mamillatum at pH values of the medium*

pH value of the medium ...	3	4	5	6	7
Mycelial weight (g.)	0	0.022	0.160	0.192	0.095

Maximum growth occurred around pH 5-6. This corresponds to the pH value of soil A. At pH values below this, growth was suppressed, so that in soil B (pH 3.7) inhibition of *P. mamillatum* could be due to this factor alone, without postulating any indirect effect of soil reaction.

DIRECT OBSERVATIONS ON *PYTHIUM* INOCULA IN SOILS

Experiment 6. Behaviour of hyphae in soils A and B. It was considered desirable to follow the behaviour of hyphae of *P. mamillatum* microscopically in soils A and B to establish further distinctions between these soils. Evidence relating to the further development or decay of *Pythium* hyphae was also sought, a feature of considerable importance, since Garrett (1956) has suggested that fungi such as *Pythium* depend largely on a rapid hyphal growth to compete with other organisms in the soil.

Glass-fibre tapes, inoculated with hyphae of *P. mamillatum*, were buried in soils A and B, which were maintained at 50 % M.H.C. in 1 lb. jam jars. Observations on triplicate tapes were made after 0, 2, 4, 7, 14 and 28 days. The controls consisted of sterilized glass-fibre tapes which had been placed on uninoculated malt-agar plates, left for 2 days, and then buried in the soils.

On tapes in soil A, hyphae of *P. mamillatum* consistently developed as follows. From 2 days onwards large numbers of oospores, chlamydospores and resting sporangia were always present, the greater proportion of the hyphal contents being utilized in the formation of these bodies. At the same time, considerable hyphal decay took place, so much so, that at the end of 1 week in soil A, very few of the original hyphae remained. Prior to the onset of hyphal decay, bacteria accumulated around the individual hyphae. This was followed by a contraction of the cell contents into irregular segments, and, finally, even these disappeared to leave only an empty hyphal shell surrounded by a sheath of bacteria. The ephemeral nature of *Pythium* hyphae is too well known to require further emphasis here, but the fact that their decay has been observed in soil, and also that bacteria are associated with the process, confirms the observations of previous workers on other fungi. It is worthy of note that the oospores produced in soil A were similar in size to those formed in culture, except that the former tended to have fewer oogonial protuberances. Oospores were invariably found at the centre of a considerable accumulation of bacterial cells and small soil particles. Warcup (1952) also found this characteristic accumulation of soil particles around oospores isolated from soil.

In soil B, the hyphae of *P. mamillatum* behaved very differently. The most significant difference was that oospores and resting sporangia were never formed. Bacterial decay of hyphae proceeded in the manner described for soil A, until, after a month, only a small quantity of irregular segments remained. Since the hyphal contents were not utilized in this soil for the production of oospores and resting sporangia, complete disappearance of hyphae seemed much slower here than in soil A. Indeed, it was still possible to find long stained pieces of hyphae after 1 week in soil B. During observations on the decay of *Pythium* hyphae in this woodland soil, hyphae of other fungi and some conidial heads were encountered. Those of *Penicillium* sp., *Trichoderma viride* and *Dicoccum asperum* were noted.

On the control tapes placed in these soils remarkably little fungal development was observed. After 2 days occasional hyphae were observed, but, subsequently, only thin actinomycete threads. This indicates that the

observations made above are not due to an antagonistic flora developing on the tapes as a result of extra nutrients carried over from the plates.

The findings from this experiment agree with those reported above, namely, that *Pythium* activity occurs chiefly during the first few days. The failure of *P. mamillatum* to develop oospores in alien woodland soil can be interpreted in two ways. First, in soil A there may be some factor stimulating oospore production, which is absent from soil B. Secondly, it is possible that the production of oospores is a normal development in soils, and that in alien soil, this normal development is prevented.

Experiment 7. Behaviour of hyphae in soils A and B amended with maize meal. In view of the striking difference between saprophytic activity of *P. mamillatum* on maize meal in soils A and B (Fig. 2), direct observations of the hyphae of this fungus were made in soils to which maize meal had been added. Soils A and B were air dried and made up to 50% M.H.C., and 2% maize meal added. Tapes containing *P. mamillatum* hyphae were buried in these soils and examined at intervals over 1 month.

In soil A, the addition of maize meal did not appreciably alter the behaviour of *Pythium* hyphae on the tapes. Oospores and resting sporangia were produced in the same abundance and with the same rapidity as on hyphae in unamended soil. During the first 2 days, however, soil particles frequently adhered to the tapes, suggesting that *Pythium* hyphae had spread on to the surrounding soil to some extent. In some instances it was possible to follow this growth microscopically on the smaller of these particles. This indication that *Pythium* is capable of growing through unsterilized soil supports the findings of H. C. Smith (cited by Garrett, 1956) who followed the growth of *Pythium* hyphae through soil over 'Cholodny' slides. It is also worthy of note that maximum activity of *P. mamillatum* hyphae occurred during the first few days after inoculation into soil, which was also indicated by the indirect studies of the previous section.

In soil B, *P. mamillatum* hyphae were not influenced by the added maize meal; decay proceeded in the manner previously described, and oospores and resting sporangia did not develop.

Experiment 8. Behaviour of hyphae in soils A and B at different moisture contents. The increased activity of *Pythium* in soils at high moisture contents has long been recognized, and this has been confirmed above. Observations of tapes buried in soils A and B at 20, 50 and 80% M.H.C. were therefore made over 1 month. Although it is difficult to obtain quantitative information from studies of this kind, hyphal decay was delayed somewhat at the higher moisture content of both soils. The formation of oospores and resting sporangia in soil A was not affected by varying the moisture content; even at 20% M.H.C. these structures were formed before desiccation of the vegetative mycelium became evident. Oospores or resting sporangia were never formed in soil B at any of the water contents tested, indicating that water availability is not the factor preventing the formation of these bodies in this soil.

Experiment 9. Behaviour of hyphae in neutralized soil B. The acid nature of the alien woodland soil has been shown to limit saprophytic activity of *P. mamillatum*, and it may therefore limit oospore production also. The behaviour of hyphae of *P. mamillatum* has therefore been followed in soil B

after neutralization; by the addition of calcium hydroxide 5 months before use, the reaction of the soil was modified from pH 3.7 to 7.1. Normal and neutral samples were air-dried and made up to 50 % M.H.C. Tapes containing hyphae of *P. mamillatum* were buried in these soils and examined at intervals over 1 month.

In neutralized soil, development contrasted markedly with that already described for acid soil B. In the neutralized soil, oospores and resting sporangia were formed very rapidly from the vegetative hyphae, and, after the first week, only these structures remained on the tapes. This behaviour is similar to that previously recorded for soil A. In acid woodland soil, then, the acid reaction seems to inhibit oospore formation. If the production of oospores by *Pythium* is regarded as a normal development in soil it seems likely that the normal life cycle of the fungus in acid soils must be severely limited, both saprophytic ability and formation of resting bodies being adversely affected.

Experiment 10. Behaviour of hyphae in sterilized sand at different pH values. The acid reaction of soil B could limit the production of oospores and resting sporangia either directly, through the fungus itself, or indirectly through some secondary effect of reaction. To establish which of these alternative hypotheses is valid, the behaviour of *Pythium* hyphae has been examined in samples of sterilized sand, maintained at different pH values. Washed quartz sand was sterilized in Petri dishes by heating to 140° C. overnight. The sand in each dish was adjusted to a pH value of 3, 6 or 7, by addition of sterilized N/10 sulphuric acid or N/10 sodium hydroxide. Tapes containing *P. mamillatum* hyphae were buried in the various dishes and examined after 1 week.

In the sand at pH 3.0, *Pythium* hyphae remained in a purely vegetative condition. In the other treatments with higher pH values, the hyphae soon developed characteristic oospores and resting sporangia. Thus the markedly acid reaction alone is sufficient to prevent *Pythium* from forming resting bodies. In the alien test soil then, the low reaction (pH 3.7) limits the parasite directly, a conclusion that has already been put forward from indirect studies of *Pythium* in soils.

DISCUSSION

The results presented above illustrate some important features of the biology of *Pythium* in soils. Colonization of fresh maize-meal by *P. mamillatum* in advance of the competing, indigenous soil flora distinguishes this parasite ecologically as a saprophytic 'sugar fungus' (Burges, 1939; Garrett, 1951, 1956). This type of fungus occurs early in the ecological succession of soil organisms which reduce organic substances to humus. Frequent reports of the isolation of *Pythium* spp. from soil on organic baits, and the demonstration by H. C. Smith (cited by Garrett, 1956), that *P. ultimum* colonized fresh sawdust added to soil, are indicative of the vigorous saprophytic potentialities of other members of the genus.

The acid conditions of 'natural' soils with pH < 5.5 may prevent the establishment and survival of *Pythium* spp. For instance, in the acid woodland soil investigated here, saprophytic activity of *P. mamillatum*, and long-

term survival by the formation of resting spores, were both prevented. The presence of the fungus in soils with neutral or alkaline reaction would seem to depend on an adequate supply of suitable material for exploitation and favourable soil conditions for this to take place. Substrates likely to be of importance include living hosts, particularly seedlings, and organic materials of high sugar content, such a range of substrates being typically found in cultivated soils. This agrees with the findings of the survey given in the first part of this paper.

I am much indebted to Prof. C. W. Wardlaw for supervising this work, and to Dr D. Park for helpful criticism.

The work was carried out during the tenure of an award from the Agricultural Research Council, to whom grateful acknowledgement is made.

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(Accepted for publication 22 July 1957)

Trans. Brit. mycol. Soc. **41** (2), 223-226 (1958).

STUDIES ON *CENTROSPORA ACERINA* (HARTIG) NEWHALL, THE CAUSE OF LICORICE ROT OF CARROT

BY S. N. S. SRIVASTAVA*

*Department of Agriculture for Scotland, Scientific Services,
East Craigs, Edinburgh*

(With Plate 9)

Symptoms of licorice rot of carrot (*Centrospora acerina*), found in Scotland for the first time, are described. The fungus tolerated up to 400 p.p.m. of boron, zinc and molybdenum and 200 p.p.m. of copper. Ultraviolet radiation of 1300 mW./cm.² induced abundant sporulation of the fungus.

Licorice rot of carrot, caused by *Centrospora acerina* (Hartig) Newhall, has been recently reported from Scotland (Srivastava, 1957). The disease was first encountered in a market garden near Edinburgh when carrots were washed clean from soil, prior to packing. A survey of the disease in April 1957, in the field where the carrots had been left over winter, showed that only one of three varieties sown there was infected. Damage was estimated to be about 5%.

This disease of carrots has hitherto only been described from U.S.A. (Rader, 1945, 1952). Neergaard & Newhall (1951) have described certain physiological characteristics of the fungus, reported it on a large number of host plants, and discussed its synonymy.

The present paper provides details of the disease on carrots in Scotland and also reports on some additional studies undertaken on the organism.

SYMPTOMS OF DISEASE

Diseased carrots showed large, blackish, rather deeply sunken lesions with an indefinite brownish, water-soaked margin. Sometimes the lesions covered the entire circumference of the roots and were 4-6 cm. long (Pl. 9, fig. 1). Often they were soft and could be easily scooped away with the thumb. Characteristic brown, torulose hyphae ramified within the affected tissues. Rader (1952) has stated that he did not notice any sporulation in nature. However, spores were found by the author on some of the lesions on carrots the day after they had been brought to the laboratory from the market garden in polythene bags.

* Colombo Plan Fellow from Directorate of Plant Protection, Quarantine and Storage, Ministry of Agriculture, Government of India.

ISOLATION, IDENTITY AND PATHOGENICITY

The fungus was isolated by trap culture from soil from the field where the carrots were grown.

It was readily isolated in pure culture on potato-dextrose agar by plating out pieces of diseased tissue from the advancing margin of the lesion. At 18–20° C., it produced an abundant, woolly, hyaline, septate mycelium, with hyphae about 4 (2.5–5) μ wide, on the surface of the medium. On the fourth or fifth day, more or less submerged light to dark olive-brown torulose hyphae, about 29 (23–35) μ in width and with cells filled with numerous oil globules, were produced. A pinkish coloration was noticed in some of the cultures, especially towards the margin and diffusing into the substratum. On the tenth day, characteristic 5–10 septate, hyaline or subhyaline, obclavate spores, 176×11 ($109\text{--}287 \times 8\text{--}13$) μ , with a prominent flat hilum, a sword-like appendage arising laterally from the basal cell of the spore, 40×2 ($20\text{--}102 \times 1.5\text{--}3$) μ and an apical, thin, tapering, whip-like beak, were observed on the surface of some of the cultures.

The pathogenicity of the isolate was proved by inoculating carrots, wounded by a pin-prick and also unwounded, with quickly growing mycelium from the edges of 3 to 4-day-old cultures (Pl. 9, fig. 2). The lesions encircled carrots kept in a damp atmosphere at about 18° C. within about 4 weeks from the date of inoculation. No spores were observed.

The isolate was found to be pathogenic to four cultivars of celery, Brydens Prize Red, Brydens Prize Pink, Giant White and Golden Self Blanching. When leaves of 2-week-old plants were sprayed with an aqueous suspension of spores and mycelium from a 10-day-old culture necrotic spots were produced within 3 days.

The fungus was reisolated from infected carrots and celery leaf spots. The controls remained healthy.

GROWTH AND SPORULATION

Several methods, which already have been used in attempts to bring about sporulation of the fungus, have been summarized by Neergaard & Newhall (1951). During the present investigations, effects of nutrition and ultraviolet radiation on single-spore cultures were studied. Duplicates were maintained and, so far as possible, all the experiments were repeated.

Growth on various media. The growth and cultural characters of the fungus were studied on potato-dextrose, oat, carrot, malt and Czapek agar at 21° C. in dark as well as in light from a fluorescent strip lamp. The fungus grew almost equally well on all media under both conditions and ultimately produced a typical colony. Cultures maintained in light showed pale or deep orange pigmentation on oat, carrot and Czapek agar, while on potato-dextrose agar a reddish coloration was noticed. No pigment production was observed in cultures kept in the dark. Neergaard & Newhall (1951) have also reported colour production to be a response to

light. Occasional sporulation was also noticed on all media except carrot-agar.

Effect of temperature. Neergaard & Newhall (1951) have reported that about 17° and 30° C. were the optimum and maximum temperatures for the growth of the fungus. Growth of the present isolate was studied at 4°, 12°, room temperature (average 18°), 21°, 26° and 30° C., with a view to detect any strain differences. It made maximum growth at 21°. The rate of growth was slower at the lower temperatures and slowest at 4°. Only slight growth was observed at 26°, though the fungus grew normally when transferred to 21° after 7 days. At 30°, no growth occurred and the fungus was killed in 6–7 days. Sporulation was occasionally observed in some of the cultures maintained at 21°. The range of this isolate was therefore somewhat lower than that described by Neergaard & Newhall.

Effect of trace element nutrition. Effects of various concentrations of zinc, boron, copper and molybdenum, ranging from 3–400 p.p.m., on growth of the fungus were studied by adding zinc sulphate, sodium borate, copper sulphate and phosphomolybdic acid to potato-dextrose agar. The pH of the medium was adjusted to 6.0, prior to sterilization as Neergaard & Newhall (1951) have reported this to be the optimum.

Even 400 p.p.m. of molybdenum, boron and zinc did not inhibit growth of the fungus although the colonies tended to be irregular. No growth occurred at this concentration of copper. Concentrations of all elements below 200 p.p.m. appeared to stimulate growth. With zinc at 3–50 p.p.m., growth was generally vigorous and regular while the torulose hyphae appeared to be rather freely formed. The diameter of the colonies on various media at the end of 7 days is given in Table 1.

Table 1. *Growth of Centrospora acerina on potato-dextrose agar containing various concentrations of boron, zinc, molybdenum and copper*

p.p.m.	Diameter of colony (mm.) after 7 days							
	400	200	100	50	25	12.5	6	3
Boron	39	37	54	53	54	56	50	50
Zinc	12	34	55	56	55	59	59	63
Molybdenum	39	51	51	53	54	60	60	63
Copper	0	21	54	54	55	57	52	50
Control	47	—	—	—	—	—	—	—

Sporulation was rarely noticed on media containing boron, but was frequent on those containing zinc, copper and molybdenum, when final observations were taken after 7 days.

Effect of ultraviolet radiation. The effect of ultraviolet radiation was mainly studied with a view to induce sporulation in the fungus. Abundant spores were produced within 48 hr. by irradiating 4 to 5-day-old cultures in Petri dishes, for 5–15 min., using a lamp giving an intensity of 1300 mW/cm.² at a distance of 18 in. Sporulation was found to improve considerably if the humidity of the cultures was maintained by covering them with a plastic sheet, immediately after removing the tops of the Petri dishes

Table 2. *Effect of ultraviolet radiation on growth of Centropora acerina on potato-dextrose agar.*

Treatment	Irradiation with ultraviolet			Control (no irradiation)
	5 min.	10 min	15 min.	
Diameter of colony (mm.) after 7 days	84	86	78	82

prior to irradiation. The diameter of the colony, 7 days after irradiation for varying periods, is given in Table 2, from which it can be seen that irradiation for even 15 min. did not depress the growth of the fungus significantly. The spores, too, germinated within about 45 min. when placed in a drop of sterile water, producing normal germ tubes.

The author is grateful to Dr C. E. Foister, Department of Agriculture for Scotland, for facilities for this work and to Mr N. A. R. Laidlaw for photographs.

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EXPLANATION OF PLATE 9

Centropora acerina on carrot

Fig. 1. Naturally infected carrots.

Fig. 2. Experimental infection of carrots, about 4 weeks after inoculation.

(Accepted for publication 25 September 1957)

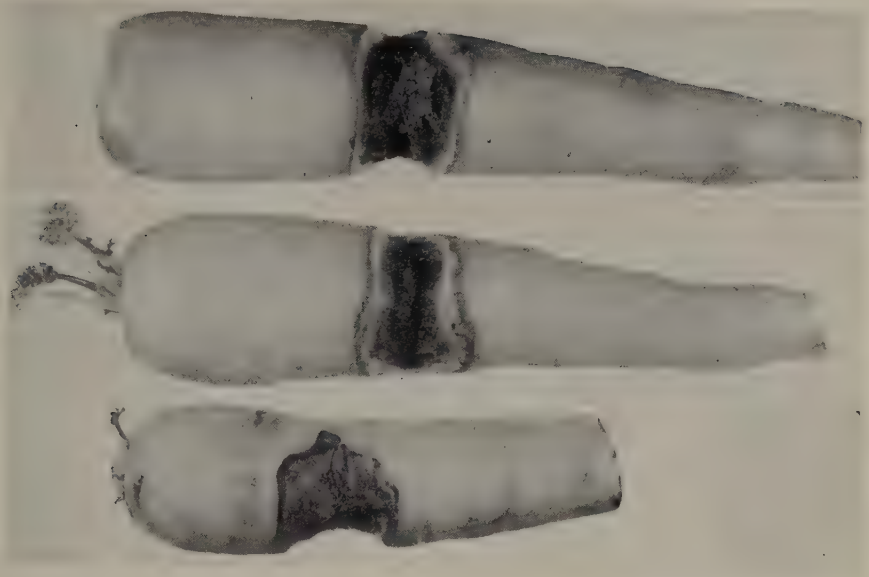


Fig. 2

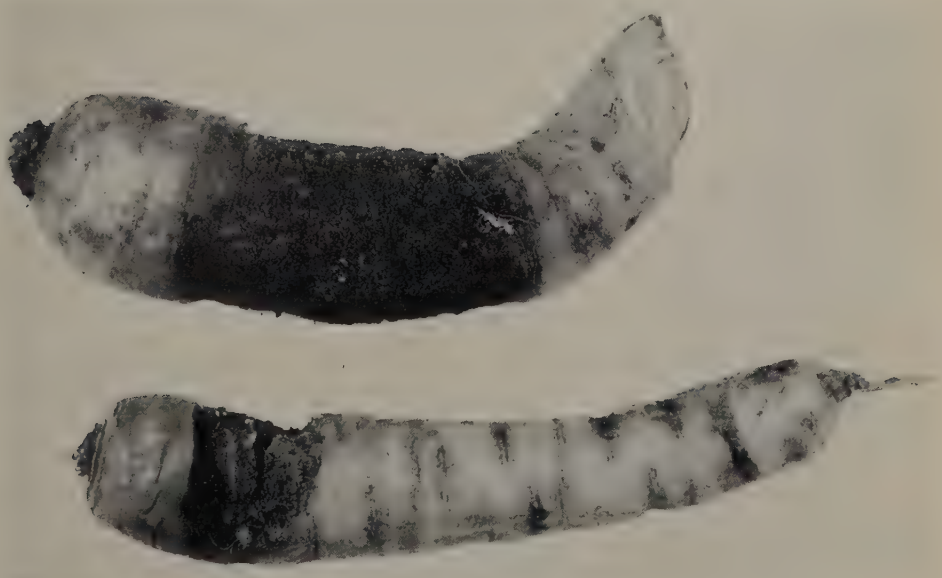


Fig. 1

SOME MECHANISMS CONTRIBUTING TO *VERTICILLIUM*-RESISTANCE IN THE HOP ROOT

By P. W. TALBOYS

East Malling Research Station

(With Plates 10 and 11)

Observations on infection of the roots of wilt-sensitive and wilt-tolerant hop cultivars by mild and virulent strains of *Verticillium albo-atrum* show that initial invasion of the host may be retarded by lignification of cell-walls and occlusion of penetrating hyphae by sheathing deposits in the epidermal and cortical cells to an extent which appears to be determined by virulence of the pathogen. Exclusion of the fungus from the pericycle and vascular tissues seems to depend upon deposition of suberin in the endodermal cell-walls, and this is associated with invasion of the cortex by the pathogen much more frequently in tolerant than in sensitive cultivars.

Though the intensity of vascular invasion appears to be regulated by these host-parasite interactions, further evidence will be required before the limits of the 'determinative' phase of disease development can be fully defined.

The defensive reactions of the host are not peculiar to the hop but are of widespread occurrence in the plant kingdom; it is suggested that their expression is related to the presence of organisms which represent a transitional stage in the evolution of specialized obligate parasites and symbiotic fungi from the primitive and essentially saprophytic forms, a sequence which is associated with a progressive delay in host-tissue necrosis and which may reflect a progressive reduction of the secretory and synthetic potentialities of the organisms.

INTRODUCTION

Experiments on the expression of *Verticillium* wilt in intervarietal graft-complexes of the hop (Keyworth, 1953; Talboys & Wilson, 1956), supported by studies of the role of fungal toxins in the development of wilt symptoms, have led to the suggestion (Talboys, 1957) that two phases of disease development can be distinguished: in the first, the determinative phase, the interactions of the mechanisms which determine resistance in the host and pathogenicity in the fungus establish within the root-system specific conditions of colonization associated either with mild or with acute symptom expression; in the second, the expressive phase, the conditions already established in the root system become apparent in terms of visual symptoms in the aerial parts of the plant.

The observations to be described provide some evidence about the nature of the host-parasite interactions which take place during the determinative phase.

The most detailed work has been mainly concerned with two commercial cultivars, the wilt-sensitive 'Fuggle', and the wilt-tolerant 'Keyworth's Midseason', the latter being commonly referred to by its original code number, 'OR/55'. Confirmatory evidence has been obtained with various experimental cultivars.

MATERIALS AND METHODS

Preparation of infected material

Studies of root invasion were made with rooted soft-wood cuttings. Small basal shoots of wilt-sensitive and wilt-tolerant cultivars were taken from established plants at the beginning of the growing season and set in a peat and sand mixture to induce the formation of adventitious roots. When roots had begun to form the plants were transferred to John Innes potting compost in 3 in. pots and allowed to remain until an extensive root system had become established. They were then moved to 4 in. pots in which the bottom layer of compost was covered with a thin layer of a sand-cornmeal culture of either a mild or a virulent isolate of *Verticillium albo-atrum*. After 10 days, and subsequently at weekly intervals, sample plants were removed and their root systems washed thoroughly. They were fixed and preserved in formalin-acetic-alcohol until required for examination. Comparable non-inoculated plants were also preserved.

Embedding and sectioning

Most of the observations were made on microtome sections of wax-embedded material. Fixed specimens were washed in 70 % ethanol, dehydrated in a series up to absolute ethanol and then transferred through three graded ethanol-chloroform mixtures to pure chloroform. Paraffin wax with ceresin (m.p. 53° C.) was added, most of the chloroform was removed by evaporation, and the specimens were then taken through two changes of pure wax with ceresin before the final embedding. By carrying out the operations with the chloroform-wax mixture and with pure wax at a relatively high temperature (63°–65° C.) for short periods, better results were obtained than with longer periods of treatment at lower temperatures. With small specimens (5 × 1 mm.) the whole process from absolute ethanol could be carried through satisfactorily within a day. In a rapid method of this kind, it is important to ensure that a high proportion of the total time is devoted to the *last* stage of each sequence, i.e. to the final treatments in absolute ethanol, pure chloroform, and pure wax, to ensure *complete removal* of the previous reagent. Thus in a typical 8 hr. sequence, the specimen would be held for 2 hr. in absolute ethanol, 2 hr. in pure chloroform, and 1 hr. in pure wax.

Sections were cut at a thickness of 5–10 μ and attached to the slides with Haupt's adhesive.

Staining

Sections were generally stained with 1.0 % aqueous safranin (10–15 min.) followed by picro-aniline blue (5–10 sec. at c. 60° C.). This combination gives particularly sharp differentiation of lignified and non-lignified tissues, provided that, after counterstaining, the sections are washed in 96 % ethanol until no more safranin diffuses out of them.

When more intense staining of thin cellulose walls was required, especially for photographic purposes, Delafields' haematoxylin was used, either

alone or as a counterstain to safranin. Both of these are basic dyes and the differentiation of tissues is therefore not sharply defined.

A saturated solution of Sudan IV was used in temporary preparations to indicate the presence of substances containing fatty acid groups, e.g. suberin; phloroglucin and hydrochloric acid were used in tests for lignin-like substances.

ROOT SURFACE COLONIZATION

Because relatively large quantities of inoculum were present it has been assumed that infection resulted mainly from the growth of hyphae directly from particles of colonized cornmeal to the surface of any roots which came into contact with them. However, the possibility is not excluded that infection might sometimes have been caused by conidia produced on the inoculum and subsequently washed into contact with the roots.

When the first samples were taken, 10 days after inoculation, small black lesions were seen extending 1–3 mm. along roots, which usually did not exceed 2 mm. in diameter. In cleared whole mounts and squash preparations of this material, it was found that the root surface was covered by darkly pigmented mycelium (Pl. 10, fig. 1), which became extremely dense in places, though it showed no evidence of three-dimensional proliferation of hyphae to form microsclerotial masses like those of *V. dahliae*. Typical verticillate conidiophores were sometimes observed, but these seemed generally to appear when the lesion had become fairly extensive and the fungus had invaded a considerable number of host cells.

There was no evidence that, under the conditions of the experiment, there were any modifications in the nature of root-surface colonization with different strains of the fungus or with different cultivars.

INVASION OF THE EPIDERMIS AND CORTEX

General characteristics

In sections of small non-infected roots stained with safranin and picro-aniline blue, the thin walls of the epidermal and cortical cells stain blue, as would be expected of a structure composed mainly of cellulose and pectic substances, but attempts by the pathogen to enter living cells in these tissues induce various modifications in the general structure and staining reactions of the cell-walls. It has been found that there is a tendency for the walls of cells in contact with the invading mycelium to become thickened and to stain with safranin, and with phloroglucin in the presence of hydrochloric acid, suggesting the presence of lignin or some similar substance (Pl. 10, fig. 2). These staining reactions have also been demonstrated in the peg-like lignitubers which arise from the *inner* surfaces of the cell-walls and are associated with the presence of mycelium on the *outer* surfaces or within the adjacent cells (Pl. 10, fig. 3).

A successful penetration through a cell-wall is almost invariably marked by a very acute constriction of the hypha, the wall being traversed by a fine 'penetration hypha', which usually does not exceed $0.5\ \mu$ in diameter.

The visual details of the process do not appear to differ essentially whether the cell-wall is of cellulose or is thickened by lignin-like deposits, though in the latter condition the penetration hypha is necessarily lengthened. It seems probable that, fundamentally, the formation of a lignituber represents a further extension of the penetration process in a wall modified by a lignin deposit; an initial partial penetration of the wall results in a further *localized* deposition of lignin which in turn is also penetrated. Repetition of this process leads to the formation of a peg-like structure, with a central canal about 0.5μ in diameter representing a greatly elongated penetration hypha.

Lignitubers are usually $2-3\mu$ in diameter at the base and may attain a length of 10μ though they are more commonly $5-8\mu$ long, tapering somewhat at the tip. Sometimes the hypha passes through the tip of the lignituber and ramifies within the cell (Pl. 10, fig. 4), but more frequently it appears to be completely occluded.

The modifications of the cell-walls result from the metabolic activity of the protoplasts within them, and appear to confer upon the host tissue some resistance to invasion by the pathogen. However, the capacity of individual cells to react to the presence of the fungus in this way is limited, and eventually, possibly as a result of the effects of fungal toxins, the cells are entered and colonized by the pathogen.

Where the host reactions are strongly developed the invaded cells tend to become very densely packed with a pseudo-sclerotial mass of darkly pigmented mycelium before invasion of the adjacent cells is effected; the edge of the invaded zone therefore tends to be sharply defined (Pl. 10, fig. 5). When the reactions are slight a relatively loose web of mycelium develops in the cells, and the invasion of the tissue is also more diffuse (Pl. 10, fig. 6).

The apparent similarity of successful penetrations through cellulose and lignified walls, together with the fact that no obvious changes take place in the physical structure or staining capacity of such walls even when penetrated by numerous hyphae, suggest that the process may be a mechanical rather than an enzymic one, for the action of an extracellular enzyme is usually apparent at a distance from its source.

In addition to the cell-to-cell type of invasion, the fungus may sometimes grow within the intercellular spaces of the cortex without immediately entering the surrounding cells. Such growth tends to be mainly in a longitudinal direction in the root, and may be sufficiently profuse to cause considerable enlargement of the intercellular spaces; the mycelium becomes pigmented, and in transverse sections presents a pseudo-parenchymatous appearance (Pl. 11, fig. 7).

There is no evidence of the *generalized* histolytic action which is commonly associated with middle-lamella destruction by extracellular fungal pectinases, and the appearance of the tissue suggests that separation of the cells may result from splitting of the middle lamella by the mechanical pressure exerted by the growing mass of mycelium in the intercellular space.

Cortical invasion in relation to fungal strain and host cultivar

The above description of the processes of epidermal and cortical invasion is of *general* application to the invasion of both sensitive and tolerant cultivars by mild and virulent strains of the pathogen. Nevertheless, there appear to be differences between the various host-parasite combinations in the *degree* to which cell-wall lignification, lignituber formation, intercellular mycelium, and intracellular colonization are developed. These differences are small and are subject to considerable variation within each combination; they are discussed below and are summarized in the first part of Table 1. It must be stressed, however, that the plants were subjected to very heavy inoculation, and it is possible that with low inoculum potentials the differences might be more clearly defined.

Sensitive host and mild pathogen. In this combination cell-wall lignification and the formation of lignitubers are seen in a pronounced form, and the invaded cortical cells become very densely packed with pigmented mycelium before invasion of neighbouring cells is effected.

Pigmented intercellular mycelium may become fairly extensive without penetrating the walls of the cells between which it passes, and in transverse sections of the root a pseudo-parenchymatous appearance develops in the enlarged intercellular spaces.

Sensitive host and virulent pathogen. Defence reactions tend to be apparent only in the early stages of epidermal and cortical invasion, and in occasional isolated cells of the middle and inner cortex. The penetration of hyphae through the tips of lignitubers has been seen most often in this host-parasite complex.

Though the first cells to become infected may contain a densely packed mass of mycelium, the majority of the invaded cells are occupied by a relatively loose web of hyphae. Intercellular mycelium is not usually obvious.

Tolerant host and mild pathogen. Host reactions are well developed, and in the early stages of infection can apparently be very effective in limiting the spread of the fungus; under these conditions a dense pseudo-parenchymatous mass of mycelium forms within and between the cortical cells. However, after growing away from the initial lesion the pathogen seems to spread fairly extensively by intercellular hyphae; subsequent invasion of the cells adjacent to these hyphae is accomplished with little or no obvious host reactions, and further generalized invasion of the cortex by a loose network of inter- and intracellular mycelium takes place.

Tolerant host and virulent pathogen. Some initial defence reaction may take place, but in general the colonization of the cortex is relatively unimpeded. A moderately dense mycelial web is formed, amongst which the host cell walls are difficult to discern, but there is usually no tendency to form pseudo-parenchymatous masses. Intercellular mycelium does not seem to take any part in extending the lesion.

It is probably significant that in both tolerant and sensitive hosts the cortical defence mechanisms seem to limit the mild pathogen more effectively than the virulent one. *The greater invasive capacity of the virulent strain seems to be associated with an increased ability to inhibit the development of the*

hosts' reactions rather than with a greater capacity to overcome them after they have occurred.

Thus the virulent strain almost invariably succeeds in colonizing the inner cortex of both sensitive and tolerant varieties, and so approaches the endodermis; the mild strain, though retarded to a greater degree in the cortex, may also be successful in reaching the endodermal layer in some roots.

INVASION OF THE ENDODERMIS, PERICYCLE AND VASCULAR SYSTEM

The main details of the host-parasite relationship in these tissues are summarized in Table 1.

The structure of the endodermal cell walls appears to be important in determining extent of fungal colonization of the tissues within this layer; as the structure undergoes important changes in the course of root growth, it is necessary first to consider these changes in non-infected roots.

Development of the endodermis

In transverse sections of a growing root, the endodermis is distinguishable about 1–2 mm. behind the tip as a uniseriate ring of regular, more or less rectangular cells separating the larger and more vacuolate cortical cells from the small primary elements of the vascular cylinder, which are in process of differentiation. The structure of the endodermal cell walls does not appear to differ from that of the cortical cells outside it.

At about the stage at which the protoxylem has become fully differentiated, it is sometimes possible to detect rather inconspicuous Casparian strips in the anticlinal walls of the endodermis (Pl. 11, fig. 8). They are more refractive than the rest of the cell wall, and stain with safranin. They do not take up Sudan IV from a saturated alcoholic solution, and it therefore appears that differentiation of the Casparian strip results from the deposition of lignin-like substances rather than of suberin.

Though it may sometimes be possible to detect suberin in the endodermal cell walls of the root-hair region, this should probably be regarded as exceptional in a 'healthy' root actively performing its absorptive functions. However, as with increasing age the structure of the root changes to accommodate the transition from a primarily absorbing to a primarily translocating function, with the development of secondary xylem and phloem and with the degeneration of the cortical tissues, increasing and sometimes very intense suberization of the endodermis takes place. The cell walls become thickened, highly refractive, and yellow tinted, while their contents may develop a brown colour which tends to be very pronounced in the nucleus (Pl. 11, fig. 9).

As the xylem and phloem tissues increase in volume the endodermal cells are stretched and eventually break up as the cortex is sloughed off. Even at this stage it is often possible to see the darkly pigmented nuclei lying in the torn endodermal cells.

Before the endodermis finally splits, a periderm is formed in the pericycle immediately within it, and the suberized cork cells which are produced

Table 1. Summary of host-parasite interactions in the hop root

	Sensitive host		Tolerant host	
	Mild pathogen	Virulent pathogen	Mild pathogen	Virulent pathogen
Epidermal reactions				
Cell-wall lignification	Well developed	Moderately developed	Well developed	Moderately developed
Lignitubers	Very frequent, not perforated	Fairly frequent, not perforated	Very frequent, not perforated	Fairly frequent, not perforated
Cortical reactions				
Cell-wall lignification	Well developed	Developed in occasional cells of mid- and outer cortex	Well developed, often sharply delimiting infected zone	Generally slight, but may develop in isolated cells
Lignitubers	Very frequent, not perforated	Fairly frequent, often perforated	Frequent, not perforated	Fairly frequent, rarely perforated
Intracellular mycelium	Dense, often pseudo-sclerotal	Loose web, or moderately dense but not pseudo-sclerotal	Pseudo-sclerotal in initial lesion; may become more sparse later	Moderately dense web, but not pseudo-sclerotal
Intercellular mycelium	May be well developed	Not usually apparent	Often well developed	Not usually apparent
Endodermal reactions				
Suberization	Occasionally developed	Occasionally developed	Usually strongly developed	Usually strongly developed
Lignification	Usually well developed	Often well developed	May augment suberization	May augment suberization
Pericycle reactions	None	None	None	None
Vascular invasion	Slight	Intense	Slight	Slight

apparently take over the function of the endodermis in providing a relatively impervious outer layer for the root.

Even in non-infected roots, particular processes do not invariably take place at the same stage of root development. Of special interest is the fact that in the wilt-tolerant cultivar OR/55, a prematurely moribund condition of the cortex in the water-absorbing zone tends to be associated with an early deposition of suberin in the endodermis, whereas in the wilt-sensitive Fuggle suberization is of much less frequent occurrence in the same circumstances; in this variety suberization may only be slight, even at the time when the endodermis begins to break down.

It seems probable that, in the hop, suberization of the endodermis is a response to degeneration of cortical tissues resulting either from normal secondary thickening of the root, or from unfavourable environmental conditions, but varietal differences may well be expressed in variations in speed and intensity of the response. It may be noted that Scott (1928) has suggested that endodermal suberization is the *cause* rather than the *result* of cortical degeneration.

The endodermis as a defence mechanism

In the tolerant cultivar. In most of the infected roots in which *either* strain of the fungus has penetrated to the inner cortex, further progress towards the vascular system has been entirely prevented by a clearly defined endodermis in which suberization of the cell-walls has taken place (Pl. 11 fig. 10). Where suberization has not become very strongly developed, it is sometimes augmented by lignin-like deposits comparable with those occurring in the cortex, but this is relatively uncommon. If invasion by the fungus is limited to a small sector of the cortex, modification of the endodermis may also be restricted to the same sector.

As a result of these changes in the structure of the cell-walls, the pathogen is only occasionally seen in the pericycle and vascular system of the roots, but, when this *does* occur, the fungus appears to colonize these tissues without inducing any lignification or suberization reactions, though small tyloses tend to occur in the vessels.

In the sensitive variety. The suberized endodermal barriers, though occasionally present, are of much less frequent occurrence, especially in small lateral roots. However, some host response takes place in the form of strong lignification of the endodermal cell-walls, but even so the pathogen succeeds in colonizing them and eventually enters the pericycle (Pl. 10, fig. 4). This defence reaction seems to be more effective against the mild strain than against the virulent, and as the mild strain is also more effectively restrained in the cortical tissues, the frequency with which it succeeds in colonizing the pericycle and vascular system is small compared with that of the virulent strain.

No defence reactions are apparent in the pericycle, and intense colonization of the tissue by the virulent strain leads to very extensive mycelial growth within the xylem elements (Pl. 11, fig. 11). Tyloses in the vessels are relatively uncommon.

There are thus varietal differences in the capacity of the host to exclude

the pathogen from the vascular bundle by means of a suberization reaction at the endodermis, and these seem to correspond with varietal differences in the amount of suberization associated with cortical degeneration in non-infected roots; it may be postulated that, in *some* hop cultivars, the cortical dysfunction which results from invasion by the pathogen leads to early suberization of the endodermal cell-walls and consequent exclusion of the fungus from the vascular system.

The endodermis as a physiological barrier

The observations indicate that in young roots the endodermis differs from the cortical tissues outside it and from the pericycle inside it in its capacity to lay down suberin in the cell-walls. It resembles the cortex and differs from the pericycle in its ability to deposit lignin-like substances in response to the presence of the pathogen.

It is thus a physiologically distinct unit forming a boundary between tissues which also differ from one another in their physiological potentialities. This is reflected, not only in the differing responses of the cells to *Verticillium* infection, but also in the mycelial characteristics of the invading pathogen.

Reference has been made to the fact that, on the root surface and within the epidermal and cortical tissues, the fungal mycelium generally becomes darkly pigmented. Though the hyphae at the advancing edge of the infected zone are hyaline, the pigmentation tends to develop rather rapidly, and it is not uncommon to find the transition from hyaline to darkly pigmented mycelium taking place within the compass of a single cortical cell.

In contrast, however, once the fungus has succeeded in penetrating the endodermis in any cultivar, the development of pigment in the mycelium is relatively uncommon. This applies not only to mycelium colonizing pericycle and phloem tissues in the young root, but also to that which, in a suitable host, succeeds in ramifying throughout the whole vascular system of the plant.

The immediate cause of pigment development in the mycelium of *V. albo-atrum* is not clear at present, though on artificial culture media it appears to be related to nutrient conditions; there is some evidence that it tends to be enhanced by the presence of certain carbon sources, including lactose and cellobiose.

Differences in the mycelial characteristics inside and outside the endodermal layer may therefore result from qualitative or quantitative differences in the supply of nutrients; it seems probable that regulation of the movement of nutrients between the pericycle and the cortex is one of the functions of the endodermis, which could thus indirectly influence the characteristics of the invading mycelium.

In the course of these investigations it has been found that, though the hyaline mycelium invariably takes up cytoplasmic stains such as aniline blue, light green, and fast green, increasing pigmentation is accompanied by a great reduction in staining capacity of the cells. If this could be interpreted as an expression of reduced permeability of the hyphal walls,

the fact may be of considerable significance, for it could then be postulated that some property of the cortical tissues induces a reduction of permeability of the hyphal walls associated with pigment development, and thus limits the secretion of fungal metabolites. This could account for the fact that though a relatively large quantity of the fungus colonizing the root cortex seems to possess very little *generalized* toxic or histolytic activity, production by the hyaline mycelium within the vascular system is probably important in the induction of aerial symptoms in the 'expressive phase' of disease development.

DISCUSSION

The observations which have been described concern the progress of the pathogen from the time at which it forms a mycelial mat on the root surface until that at which it enters the vascular system. They show that invasion of the host may be slowed down by 'lignification' reactions at the epidermis and cortex to an extent which appears to depend on the virulence of the pathogen, and that, in some cultivars, invasion of the vascular system can be greatly reduced by suberization of the endodermal cell-walls.

Throughout the investigation, the sharply discontinuous distributions of the fungus within the tissues, which suggest the presence of local defence reactions, have invariably been associated with one or other of these modifications in cell wall structure. There is no *visual* evidence of the existence of other defence mechanisms, though a slight and *uniform* reduction in the level of infection throughout a tissue, resulting from the action of a uniformly distributed factor inhibiting growth of the fungus or secretion of its metabolites, would not necessarily be detected.

Differences in intensity of vascular invasion of the infected roots, which result from interaction of various cultivars with mild and virulent strains of the pathogen, appear to be directly related to intensity of the symptoms which subsequently develop in aerial parts of the plants; but although the cortical and endodermal reactions seem to determine the intensity of vascular invasion, it cannot immediately be assumed that the host-parasite interactions in these tissues comprise the whole of the determinative phase of disease development. It must be noted, for example, that the observations do not take into account the possibility that further defensive reactions might operate within the vascular system of the host. In the absence of such reactions, there seems to be no reason why the small amounts of mycelium which succeed in entering the vessels of roots of tolerant cultivars should not proliferate there and become as intense as that in sensitive cultivars. The occurrence of small tyloses in the primary vessels of some roots after the initiation of vascular invasion has been noted; the possible significance of these structures as a defensive mechanism will be discussed elsewhere.

The observations on root invasion have also taken no account of the processes which lead up to the establishment of the mycelium on the root surface, and which are likely to be affected by such factors as the exudation of substances from the roots and the saprophytic potentialities of the fungus in the soil. Until more information is available on the events

preceding root surface colonization, and on those following the initial vascular infection, it will not be possible to define more closely the limits of the determinative phase.

The various defence reactions occurring in the *Verticillium*-hop complex are not uniquely associated either with the particular host or with the particular pathogen but have been reported, severally or collectively, and with minor variations, in a number of parasitic and symbiotic conditions. The protective effect of a suberized endodermis has, for example, been demonstrated by Voorhees (1934), who found that the resistance of some cultivars of corn to the causal organisms of 'seedling blights', *Gibberella moniliformis* and *G. zeae*, was related to the degree of suberization of the endodermis in the mesocotyl and primary radicle; Butler & Jones (1949) refer to the fact that *Fusarium nivium* is unable to penetrate the suberized endodermis in the water-melon root, and can only enter the vascular system by invading the root-tip zone before suberization has taken place.

In many mycorrhizal associations, the invading fungus is invariably restricted to the tissues lying outside the endodermis, though it seems improbable that *generalized* suberization is important in truly symbiotic associations, as it would presumably isolate the mycorrhizal tissues from the vascular system and thus from the rest of the plant. However, according to Butler & Jones (1949), restriction of *Rhizophagus*-type endophytes in orchids is due to the presence of the suberized Casparian strip in the endodermis.

Reports of the limitation of fungal invasion by a suberized endodermis generally suggest that this condition is one inherent in the root structure at the time of infection, rather than one prematurely induced by presence of the fungus in the cortex, as suggested above.

The term 'lignituber' was suggested by Fellows (1928), in his account of the invasion of wheat roots by *Ophiobolus graminis*, to describe structures which appear to have been identical with those found in *Verticillium*-infected hop roots, and those reported by Van der Meer (1925) in cucumber and potato roots invaded by *V. albo-atrum*. The term is properly applied to such structures only when the sheathing material is a lignin-like substance; others in which the sheath is of cellulose, callose, suberin, or cutin are preferably described as 'infection pegs'.

It is generally considered that all such structures represent a defensive reaction by the invaded cell, though Corner (1935) believed that infection pegs formed by *Erysiphe graminis* resulted from swelling of the cellulose walls of the host following enzyme action by the penetrating hyphae. He suggested that failure to penetrate the peg in resistant plants was an expression of a toxic action by the host cytoplasm.

Jeffrey (1898) described the occurrence of 'cutinized sheaths' around hyphae penetrating the rhizoids and epidermal cells of gametophytes of *Botrychium virginianum* in the course of development of a mycorrhizal condition; thickened and lignified cell-walls and lignified papillae have been noted by Kusano (1911) in the unusual mycorrhizal association between *Armillaria mellea* and the non-chlorophyllous orchid *Gastrodia elata*; he refers also to the fact that structures resembling the papillae have been reported by other authors in mycorrhizas of *Botrychium*, *Lycopodium*,

Lecanorchis, *Calypogeia*, *Neottia* and *Coffea*. The occurrence of cell-wall thickening as a defensive mechanism in orchid mycorrhizas has similarly been reported by Burges (1939); Young (1926) found that seedlings of certain plant species normally resistant to attack by *Alternaria*, *Helminthosporium*, *Cephalosporium* and *Diplodia* spp. became infected and produced well-defined infection pegs ('callosities') if grown under reduced illumination.

The present writer has observed cell-wall lignification and lignitubers in tomato roots infected by *Colletotrichum atramentarium*.

Blackwell (1953) has reported occurrence of lignified cell walls in potato tubers infected by *Phytophthora infestans* and has shown that the simple filiform haustoria produced by this species may sometimes become sheathed and occluded by a thickening of the cell wall. This reaction did not affect all haustoria in a particular tuber, and was never observed in the mesophyll cells of infected leaves. The description and illustrations of the sheathed haustoria suggest a close resemblance to the infection pegs recorded in other host-parasite associations.

The extensive study by Fraymouth (1956) of haustoria in the higher Peronosporales has demonstrated that their entry into the cells of many host species is accompanied by deposition of sheaths, generally of callose. No reference is made to the occurrence of thickening or lignification of the cell-walls, or to lignification of the haustorial sheaths, but, in incidental observations made by the present author, it has been found that in the pith and cortex of hop shoots infected with *Pseudoperonospora humuli* the host cell-walls in contact with the intercellular mycelium become greatly thickened and lignified (Pl. 11, fig. 12); the haustoria, though initially sheathed with callose or cellulose, eventually become lignified.

Though the apparent similarity of the host reactions which lead to cell-wall modifications and to the formation of lignitubers, infection pegs, and haustorial sheaths suggests the possibility that these responses may be homologous, the varied chemical composition of the resulting deposits may indicate analogous rather than homologous development.

At first sight the occurrence of possibly homologous processes in taxonomic groups ranging from lycopods to angiosperms may be a matter of surprise, but it is clear that the ability to resist fungal attack must have been an important feature of autotrophic plants continuously from an early stage in their evolution, and an effective defence evolved in primitive plants would have been likely to persist in some form irrespective of evolutionary divergence in other processes. Such active defence mechanisms may contribute very significantly to the fact that most plants are resistant to most fungi.

It seems probable that the various reactions occur as a result of the presence of metabolites secreted by the invading fungi; but if the activity or the concentration of such substances is excessive, necrosis of the affected host cells may take place before any response has occurred; alternatively, a very low level of secretory activity may fail to induce a response.

In very general terms it may be postulated that in the course of evolution from the saprophytic to the parasitic habit, independently in various distinct groups of fungi, the primitive pathogens were usually

grossly lysigenous and necrogenous organisms which, having entered the host by colonizing moribund or dead tissues, killed the host cells too rapidly to allow any defensive response to occur. Subsequent development of effective penetrative mechanisms may have eliminated the necessity for preliminary colonization of tissues damaged in other ways, though the 'action in advance' of the enzymes and toxins secreted by the organism enabled it to remain essentially saprophytic.

In a transitional stage, progressive reduction in the secretory activity of the pathogens, leading to a corresponding postponement of host-cell necrosis, permitted the expression of the hosts' defensive mechanisms and resulted in a limitation of colonization by the pathogens. This condition is exemplified by the *Verticillium*-hop root complex, and in varying degrees by the other host-parasite associations which have been discussed above.

The evolutionary sequence appears to have culminated in the development of organisms in which secretory activity was reduced to such a low level that necrosis of the host was long-delayed or absent, and the presence of the fungus around and even within the host cells could be tolerated with little or no visible response; but a reduction in the secretion of extracellular enzymes and toxins would at once limit the range of nutrients potentially available to the parasite. Instead of breaking down a variety of substrates to easily assimilated substances, it would be forced to rely upon the supply of directly assimilable materials already available. Furthermore, the complex integration of enzyme systems in the living cell, and the possibility of reversal of enzyme action under suitable conditions, suggest that the elimination of extracellular lytic enzymes might immediately entail losses of synthetic capacity in the intracellular system.

However, if substances supplied by the host could immediately compensate for such losses, a selective advantage would be conferred upon the pathogen in respect of competition with more necrogenous forms; such a situation could lead to a high degree of host-parasite specificity, and could also provide a basis for the development of a *mutually* advantageous association.

This general concept may be related directly to the sequence, facultative parasite—facultative saprophyte—obligate parasite, which Yarwood (1956) has illustrated by reference to *Phytophthora citrophthora*, *P. infestans*, and *Peronospora destructor*; and in this context it is interesting to note that Fraymouth (1956) has described the association of *P. chlorae* with *Blackstonia perfoliata* as '... a balanced relationship, almost mycorrhizal in nature...'.

Applying the concept to the evolution of the mycorrhizal habit, the sequence is substantially similar to one suggested by Garrett (1956) and is consistent with his opinion that the essential difference between primitive root parasites and mycorrhizal organisms lies in the greater 'destructive disorganization' of the host tissues by the pathogenic forms. That the mycorrhizal condition results from reduced synthetic or secretory capacity, or both, is suggested by the evidence of Melin (1948) and others, who have shown that whereas litter-decomposing fungi can utilize cellulose or lignin, mycorrhizal forms are frequently unable to do so. Similarly, some mycorrhizal fungi have lost the ability to synthesize various vitamins.

Little is known at present about the secretory potentialities of *V. albo-atrum*, but its behaviour within the root tissues of the hop, justifying its inclusion in the 'transitional' group of pathogens postulated above, suggests that appreciable limitation of secretory activity has occurred; though it appears that in the virulent strain the process of specialization has been reversed and has led to the re-establishment of a more primitive condition, associated with increased suppression of the host's cortical defence reactions.

The evidence which has been presented suggests that an important part in the defence of the hop against *V. albo-atrum* is played by physiological responses which are probably not specifically associated with, and were not necessarily evolved in the presence of, this particular pathogen; their operation would help to exclude any organism which, in attempting to enter the host, provides the stimulus required to initiate them.

Certain facts in the history of hop wilt support this conclusion. Of the wilt-tolerant cultivars at present grown commercially on infected land, Keyworth's Midseason (OR/55), Bramling Cross (OT/48), and Keyworth's Early (OJ/47) were bred at Wye College in East Kent in 1924, 1927, and 1930 respectively, and Whitbread's Golding Variety (1147) was bred on a commercial farm in the Paddock Wood area, east of Tonbridge, about 1911.

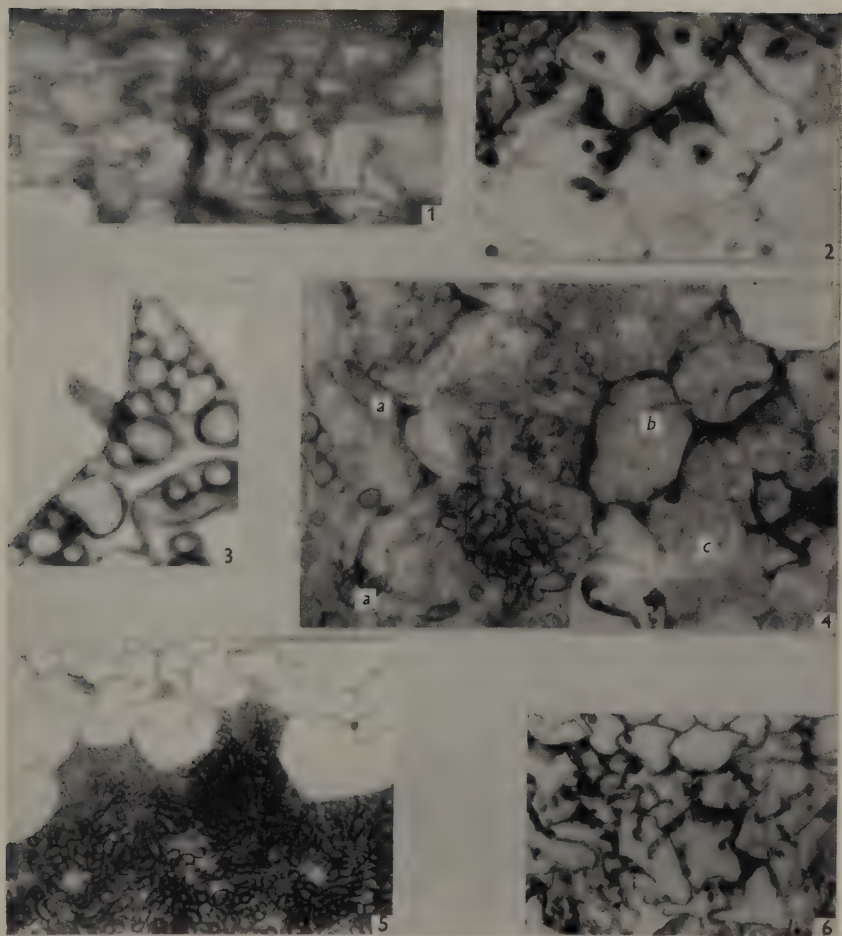
The first record of *Verticillium* wilt in hops was made in 1924 (Wormald, 1925) at Penshurst, near Tunbridge Wells; but this was a relatively mild disease, corresponding with what is now known as 'fluctuating' wilt (Keyworth, 1942). There was no evidence of a more acutely pathogenic form of the disease until about 1930, when a new variant, the very lethal 'progressive' wilt, appears to have arisen on a farm in the Paddock Wood area. It has subsequently spread to many more farms, but, until recently, the hop-growing areas of East Kent had remained free from the disease. At Wye College *Verticillium* wilt of hops was recorded in 1954 for the first time (Darling 1955).

Thus the Wye cultivars which subsequently proved to be wilt-tolerant were bred 20-30 years before any form of the disease had been recognized in the gardens in which they originated, and *probably before 'progressive wilt' existed*. The partial American ancestry of this group of tolerant cultivars seems to offer no immediate explanation, as the ancestral cultivar concerned, *Humulus lupulus* var. *neo-mexicanus*, appears to be wilt-sensitive; furthermore, Whitbread's Golding Variety (1147) is believed to be of purely European parentage.

The author wishes to record his indebtedness to Miss D. P. Laurence, for extensive technical assistance in the course of this investigation, and to Miss J. M. Hay and Mr E. Y. Jones for the preparation of photomicrographs.

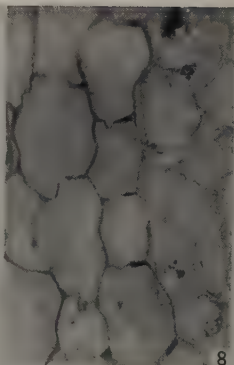
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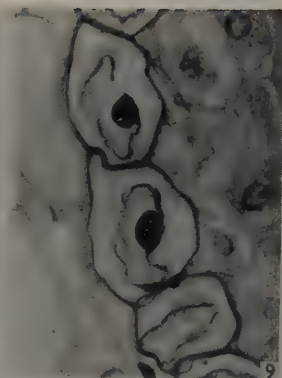




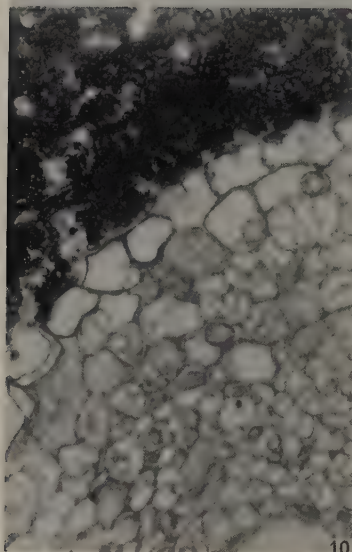
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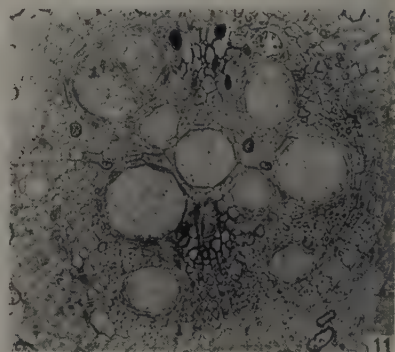
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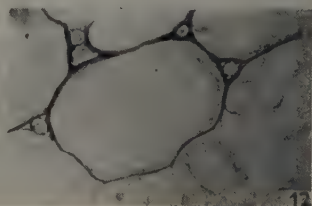
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EXPLANATION OF PLATES 10 AND 11

PLATE 10

- Fig. 1. Mycelium of *V. albo-atrum* on the surface of a hop root. Cleared whole-mount.
- Fig. 2. Longitudinal tangential section of root invaded by *V. albo-atrum* showing thickened lignified cell-walls and (out of focus) lignitubers: mycelium in radially adjacent cells had been removed in earlier sections.
- Fig. 3. Typical lignituber, with central canal apparently continuous with the underlying hypha.
- Fig. 4.* Transverse section of root of wilt-sensitive cultivar (Fuggle) invaded by virulent strain of *V. albo-atrum*: (a) two lignitubers perforated by hyphae in the cortex; (b) colonized endodermal cells with lignified walls; (c) invaded pericycle and vascular tissues.
- Fig. 5. T.S. root cortex showing sharp delimitation of invaded tissue and pseudosclerotial mass of mycelium associated with strongly developed defensive reactions.
- Fig. 6. T. S. root cortex showing diffuse distribution of mycelium associated with poorly developed defensive reactions.

PLATE 11

- Fig. 7. T.S. cortical cells with pigmented mycelium in the enlarged intercellular spaces.
- Fig. 8. T.S. near tip of non-infected root, showing lignified Casparian strips in radial endodermal walls.
- Fig. 9. T.S. non-infected root of tolerant cultivar (OR/55) with very intense endodermal suberization and discoloration of cell contents associated with cortical degeneration.
- Fig. 10. T.S. root of a tolerant cultivar (OR/55) showing obstruction of a virulent strain of *V. albo-atrum* at the endodermis.
- Fig. 11. T.S. root of a sensitive cultivar (R2/25a) with intense vascular colonization by a virulent strain of *V. albo-atrum*.
- Fig. 12. Thickened lignified cell walls associated with intercellular mycelium of *Pseudoperonospora humuli* in the pith of a hop stem.

* Composite photograph, combining two adjacent fields of view.

(Accepted for publication 27 August 1957)

DEGRADATION OF CELLULOSE BY *VERTICILLIUM ALBO-ATRUM*

BY P. W. TALBOYS

East Malling Research Station

(With Plate 12 and 5 Text-figures)

The production of a cellulase system, which reduced the viscosity of methyl cellulose solutions and broke down cellulose to form reducing-substances, took place in liquid media containing cellulose or cellobiose as the sole source of carbon. Secretion of the enzyme in the presence of cellulose was inhibited by dextrose, sucrose, starch and lactose. Sodium nitrate formed a suitable nitrogen source over a wide range of concentrations, but peptone was inhibitory at or above a concentration of 0.5 %. Studies of the colonization of Cellophane membranes showed that degradation was progressively reduced in the presence of increasing concentrations of dextrose, even though some delamination still took place.

It is suggested that the presence of sugars, starch, etc., in root tissues invaded by *V. albo-atrum* inhibits cellulase production and so prevents generalized cell-wall destruction, even though very small amounts of cellulase may still aid cell-wall penetration by the pathogen.

INTRODUCTION

In the course of observations on invasion of the epidermis and cortex of the hop root by *Verticillium albo-atrum* (Talboys, 1958) it was found that, though these tissues were often intensively colonized by the pathogen, there was no evidence of a general breakdown resulting from the secretion of histolytic enzymes such as pectinases and cellulases. The evidence suggested that passage of the very fine 'penetration hyphae' through cell-walls was accomplished by mechanical rather than by chemical means.

Subsequent studies of the penetrative potentialities of the fungus showed that when a spore suspension was placed on a cellulose ('Cellophane') membrane, 40 μ in thickness, lying on the surface of a plate of Dox's agar, the mycelium readily penetrated the membrane and entered the nutrient layer beneath. After about 10 days, the membrane could no longer be stripped intact from the agar, because in the region of the original inoculum the Cellophane had lost all coherence. The immediate inference that enzymic degradation might have taken place was strengthened by evidence that the fungus would grow and sporulate satisfactorily on synthetic media in which filter-paper pulp or cotton-wool provided the sole source of carbon.

A potentiality for cellulase production by the pathogen is clearly of considerable significance, not only in relation to the processes of host invasion but also in relation to its persistence in organic matter in the soil. A short investigation with both mild and virulent strains of *V. albo-atrum*, to be

described below, has provided direct evidence of the production of a cellulase system, has indicated some of its properties, and has demonstrated how its secretion may be influenced by the nature of the available carbon and nitrogen sources.

CELLULASE PRODUCTION IN A SYNTHETIC MEDIUM

Readily detectable quantities of cellulase were produced when the fungus was grown at 23°–25° C. at pH 5.8–6.0 in a liquid culture of the following composition: filter-paper 1.0 %, NaNO_3 0.2 %, KH_2PO_4 0.1 %, MgSO_4 0.05 %, KCl 0.05 %, FeSO_4 0.001 %, distilled water to 100 %. The cultures were grown in 500 ml. Erlenmeyer flasks, each containing 50 ml. medium, and one 9.0 cm. No. 1 Whatman filter-paper was added to each 50 ml. portion of solution separately and dispersed by means of a macerator before sterilization.

The flasks were each inoculated with a drop of a dense spore suspension, and some cellulase activity was usually detectable 5 days later. For assay and for other experiments, the fungal mycelium and spores and the excess filter-paper pulp were removed by centrifuging the suspension at 4500 r.p.m. for 10 min.

Detection and assay of cellulase

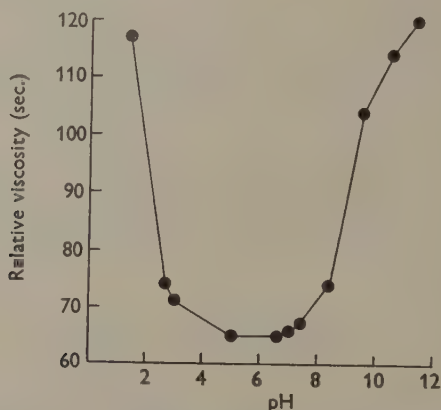
Action on methyl cellulose. Cell-free preparations derived from liquid cultures more than 5 days old were found to reduce the viscosity of a methyl cellulose solution; autoclaved samples induced no change of viscosity. This property formed the basis of a convenient technique for routine detection and assay of the cellulase system. For comparative purposes, 5 ml. enzyme solution were added to 10 ml. 0.75 % aqueous methyl cellulose (B.D.H.) and the mixture immediately transferred to a No. 2 B.S.I. Ostwald viscometer maintained at 20° C. in a water-bath. The time (in seconds) required for a standard volume of the mixture to pass through the capillary of the instrument was taken as a measure of the relative viscosity. The first reading was taken as soon as possible after mixing the solutions, and subsequent readings at 5 min. intervals for about 20 min. Comparisons of the activities of various solutions were made either by determining their relative viscosities after a standard period of treatment, or by examination of graphs showing the changes of relative viscosity with time over a period of 20–25 min.

The action of the enzyme on methyl cellulose takes place over a fairly wide range of pH (Text-fig. 1), but the optimal value is about 5.6–5.8, and tests have generally been carried out in that range. There is little evidence to indicate the chemical action of the enzyme on methyl cellulose, but tests with Fehling's solution have suggested that no reducing sugars are formed.

Action on cellulose. Incubation of active cellulase preparations with filter-paper pulp at 25° C. for 2–3 days in the presence of toluene leads to formation of appreciable quantities of reducing substances. These have not been positively identified, but they react with phenylhydrazine in the

presence of acetic acid to form an osazone, which precipitates only when the reaction mixture has cooled. In this and in the microscopic appearance of the aggregates of crystals, it differs considerably from glucosazone and somewhat resembles the osazone of cellobiose.

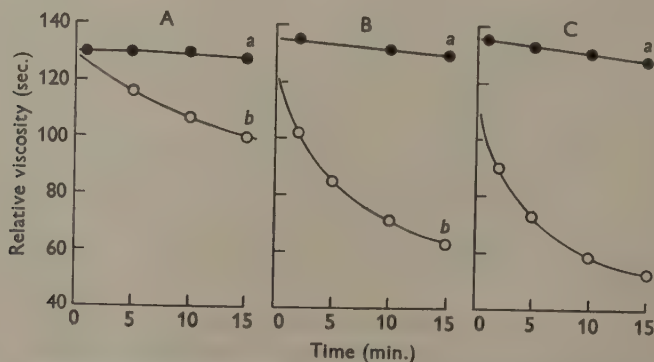
Comparison of the effects of the preparations on cellulose and on methyl cellulose suggests that a system is present comparable with that indicated by Siu (1951), in which an initial cleavage of 3-dimensional linkages in the cellulose molecule is brought about by a 'cellulase', while another enzyme referred to as 'Cx' hydrolyses the β -glycosidic linkages of non-methylated linear polysaccharide chains.



Text-fig. 1. Effect of pH on the degradation of methyl cellulose by a crude cellulase preparation

Effect of added carbohydrates

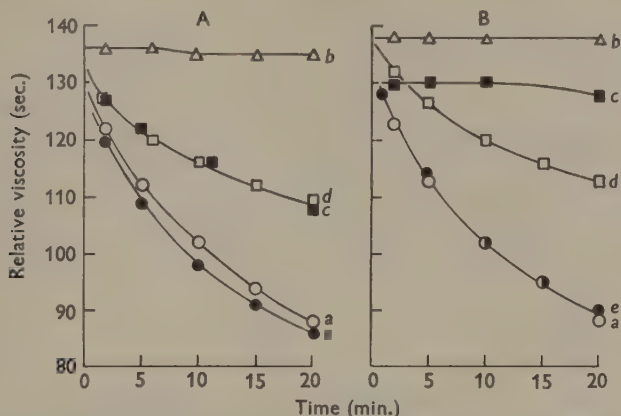
Though fairly active preparations of cellulase were obtained in the filter-paper medium, the growth of mycelium was rather sparse. The addition of 1% dextrose to the medium resulted in formation of a dense mat of mycelium, but cellulase production was reduced to a very low level. Text-fig. 2 illustrates the comparative cellulase activity in cultures



Text-fig. 2. The reduction of viscosity of methyl cellulose solutions by filtrates from cultures (a) with and (b) without dextrose, after 5 days (A), 7 days (B), and 8 days (C).

of a mild strain of *V. albo-atrum*, with and without added dextrose, 5, 7 and 8 days after inoculation. At the end of this period, the dextrose had been completely utilized and some cellulase activity had developed. Similar results were obtained with a virulent strain of the pathogen.

In comparable experiments, the effects of added starch, sucrose, lactose and cellobiose were determined, and the results of assays made 6 days after inoculation are shown in Text-fig. 3.



Text-fig. 3. Cellulase production by virulent (A) and mild (B) strains of *V. albo-atrum* in the presence of cellulose (a), and with the addition of sucrose (b), lactose (c), starch (d), and cellobiose (e), indicated by the change in viscosity of methyl cellulose solutions.

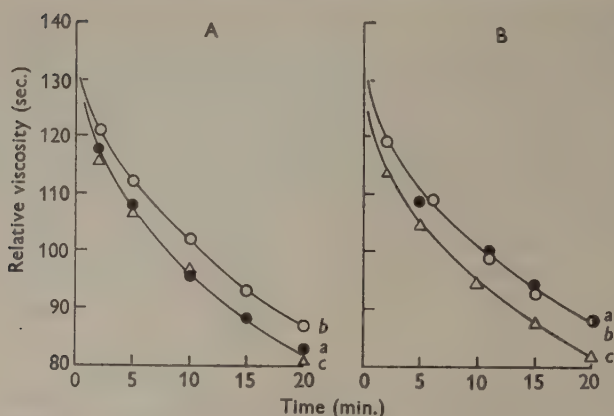
Starch, sucrose and lactose all caused clearly defined reduction in cellulase secretion, though the effect was less marked in both strains in the presence of starch, and in the virulent strain in the presence of lactose; it may be noted, however, that at the time of assay all of the starch and almost all of the lactose had been utilized, whereas there were appreciable residues of sucrose and cellobiose. The apparent difference between the mild and virulent strains in respect of the action of lactose was not maintained in subsequent experiments.

Cellobiose caused no inhibition of cellulase secretion, and later the enzyme was found to be produced as readily in media in which cellobiose was the sole carbon source as in cellulose media.

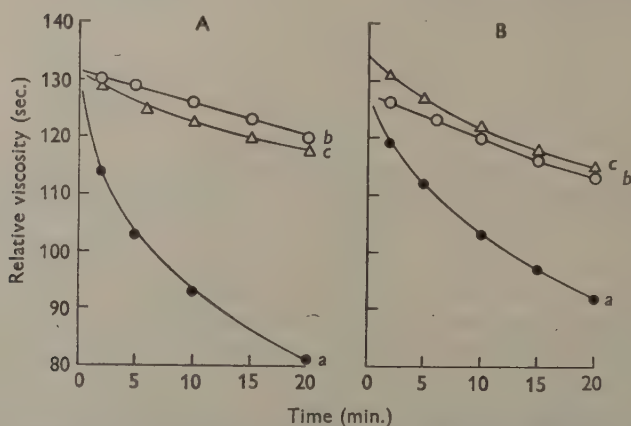
Influence of the nitrogen source

Cellulase production has been determined in filter-paper media containing either sodium nitrate or peptone as the source of nitrogen at concentrations of 0.1, 0.5, and 2.0 %. The results are shown in Text-figs. 4 and 5.

The concentration of sodium nitrate appeared to have little or no influence on intensity of cellulase secretion by either strain of the pathogen within the range used in the experiment; but in both strains the higher concentrations of peptone caused a marked reduction in cellulase activity, though mycelial growth was more profuse in these than in any of the other media.



Text-fig. 4. Cellulose production by virulent (A) and mild (B) strains of *V. albo-atrum* in the presence of 0.1% (a), 0.5% (b), and 2.0% (c) sodium nitrate.



Text-fig. 5. Cellulose production by virulent (A) and mild (B) strains of *V. albo-atrum* in the presence of 0.1% (a), 0.5% (b), and 2.0% (c) peptone

Pectolytic action of cellulase preparations

An enzyme capable of reducing the viscosity of pectin solutions without causing an increase in the amount of reducing substances present, a pectin depolymerase, was identified in all the liquid cultures. Its formation was thus independent of the presence of pectin, and it was not suppressed by the nutrient factors which suppressed cellulase production.

DEGRADATION OF CELLULOSE MEMBRANES

The influence of nutrient supplies on the capacity of the fungus to degrade and utilize cellulose has been further demonstrated by observations on the colonization of Cellophane membranes. Sterile Cellophane disks were placed on the surface of plates of modified Dox's agar containing 0, 0.01, 0.1, and 1.0% dextrose, and were inoculated at the centre with a drop

of spore suspension. After 7 days the membranes were carefully stripped from the agar surface and fixed in formalin-acetic-alcohol. A segment of each colony was then removed, embedded in paraffin wax, and sectioned vertically along a radius, so that early stages of penetration could be seen at the edge of the colony and later stages towards the centre.

The results were consistent with those obtained in the experiments on the effect of dextrose on cellulase secretion in liquid culture; in the absence of dextrose, and at the lowest concentration, the cellulose layer was completely decomposed at the centre of the colony and, in that region, only a mat of mycelium could be stripped from the agar surface; at the highest concentration, the fungus had colonized the cellulose very extensively, splitting it into laminae, but only in the upper part of the membrane was there evidence of complete dissolution. The effect of colonization of the membrane without degradation was to increase its total thickness, whereas at low concentrations of dextrose rapid dissolution led to a great reduction of thickness. Pl. 12 fig. 1, shows the first stage of colonization of the membrane near the edge of a colony on 0.1 % dextrose medium. Pl. 12, figs. 2-4, illustrate the degree of colonization and degradation of membranes on 0.01, 0.1 and 1.0 % dextrose media. Each of these sections illustrates the condition of the membrane at approximately half way along a radius of the colony.

Greater complexity of the underlying medium reduced the degradation of the cellulose layer still further, and Pl. 12, fig. 5, shows the colonization of Cellophane over prune-extract agar augmented with 0.5 % lactose and 0.1 % yeast extract.

DISCUSSION

No attempt has been made to investigate at all closely the chemical mechanisms underlying the action of the cellulase system of *V. albo-atrum* under various conditions, but sufficient evidence has been obtained to give some indication of the possible significance of the system in the parasitic and saprophytic activities of the organism.

The suggestion that certain root tissues might possess the capacity to induce physiological changes affecting the secretory activity of the pathogen has been made as a result of histological studies of invaded roots (Talboys, 1958). The experiments described above appear to confirm that such modifications are possible, for although the mechanism of the action is not apparent there can be little doubt that within the root tissues of the host, in the presence of sugars, starch, and other organic substances, the production of cellulase must be considerably curtailed.

Though the evidence thus provides a partial explanation of the fact that a general breakdown of cell-walls does not occur in invaded root tissues even in the absence of lignification reactions, the possibility remains that the secretion of very small quantities of cellulase might still aid cell-wall penetration by the pathogen; the evidence does not account for the very limited extent of pectolytic breakdown of middle lamella material.

The cellulase system seems, therefore, to be of minor importance in the pathological phase of the organism, but after death of the host, when the

fungus enters upon a saprophytic phase of existence, it is probable that the ability to utilize cellulose permits a considerable prolongation of active growth after the more readily assimilable substrates have been exhausted. Melin (1948) has indicated that whereas the essentially saprophytic litter-decomposing fungi are able to utilize cellulose and lignin, mycorrhizal forms are frequently unable to do so. The nature of the cellulase system of *V. albo-atrum* seems to confer upon the organism some of the characteristics of both groups, and perhaps strengthens the view (Talboys, 1958) that the fungus represents a transitional stage in the evolution of the parasitic habit.

The author is indebted to Miss D. P. Laurence for technical assistance, and to Mr E. Y. Jones for the preparation of the photomicrographs.

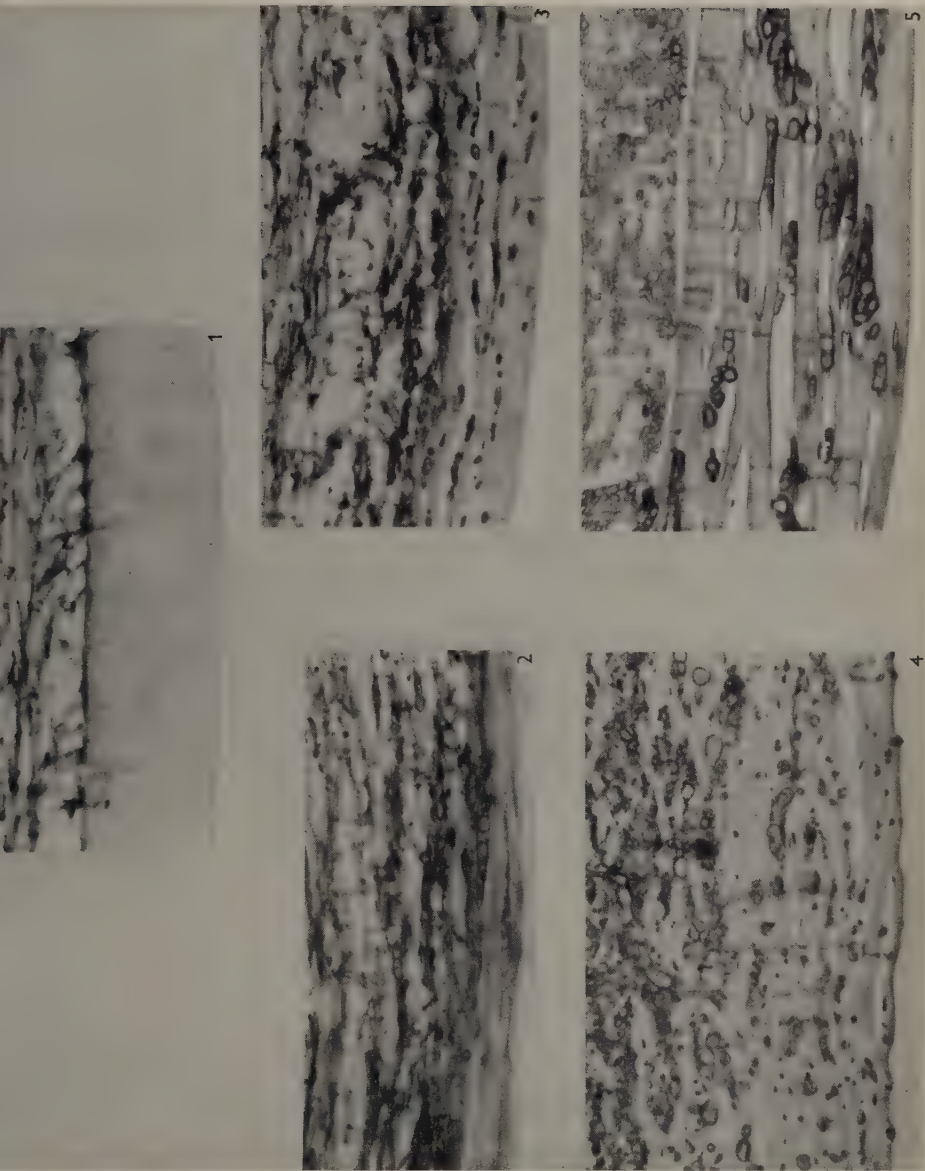
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EXPLANATION OF PLATE 12

- Fig. 1. Radial vertical section near the edge of a colony of *V. albo-atrum* on Cellophane, showing initial penetration into the membrane.
Figs. 2-5. Radial vertical sections at approximately half-way along colony radii, showing relation between membrane dissolution and composition of underlying agar medium.
Fig. 2. 0.1% dextrose present.
Fig. 3. 0.10% dextrose present.
Fig. 4. 1.00% dextrose present.
Fig. 5. Prune extract with 0.5% lactose and 0.1% yeast extract present.

(Accepted for publication 27 August 1957)



ASSOCIATION OF TYLOSIS AND HYPERPLASIA OF THE XYLEM WITH VASCULAR INVASION OF THE HOP BY *VERTICILLIUM ALBO-ATRUM*

By P. W. TALBOYS

East Malling Research Station

(With Plates 13 and 14 and 1 Text-figure)

In *Verticillium* wilt of the hop, acute symptoms are usually associated with intense mycelial colonization of the vascular system and slight tylosis; mild symptoms are associated with sparse mycelium and intense tylosis, accompanied by prolonged or renewed cambial activity leading to xylem hyperplasia. These reactions, which seem to correspond with the traumatic responses of the plant, may also be induced by other pathogens.

Histological evidence suggests that leaf necrosis results from the action of fungal toxins, and that the processes leading to necrosis are essentially similar in the mild and acute syndromes.

It is suggested that the determinative phase of disease development ends when the intensity of the primary vascular invasion, which follows the host-parasite interactions in the extra-vascular tissues, determines whether the formation of tyloses will be stimulated or suppressed; either condition, once established, tends to be self-perpetuating.

INTRODUCTION

Studies of the role of fungal metabolites in the development of the hop wilt syndrome have led to the conclusion that, though toxin production may play a part in the development of leaf symptoms, variations in the intensity of symptom expression are likely to be determined by the *quantity* and *distribution* of the fungus within the plant rather than by differences in the inherent toxigenicity of the pathogen and the toxin-sensitivity of the host (Talboys, 1957). Some evidence in support of this view has been obtained from observations on the processes of root invasion, which have suggested that defensive reactions occurring in the cortical and endodermal tissues of the host are important in determining the intensity of the *initial* colonization of the vascular system by the pathogen (Talboys, 1958); the *subsequent* host-parasite interactions, which ultimately lead to the expression of symptoms in the aerial parts of the plant, form the subject of the investigations to be described in this paper.

THE DEVELOPMENT AND DISTRIBUTION OF TYLOSES

Many plants of both wilt-sensitive and wilt-tolerant hop cultivars infected with various strains of *Verticillium albo-atrum* have been examined anatomically. They have included plants infected by natural means in commercial hop-gardens, as well as others which had been subjected to

heavy inoculation in experimental plots, e.g. in varietal tolerance trials, as described by Keyworth, Hitchcock, & Goode (1953) and in pathogenicity-testing experiments (Talboys & Wilson, 1954).

Large specimens of roots and stems, either fresh or fixed in formalin-acetic-alcohol, were sectioned directly, either by hand or by sledge microtome. Small roots and leaf tissues were embedded in wax by an ethanol-xylene technique before sectioning. Temporary preparations were stained with cotton blue in lactophenol, permanent preparations either with safranin and picroaniline blue or, occasionally, with basic fuchsin and light green.

The structure of the xylem in the hop is such that most of the vessels are adjacent to a number of xylem parenchyma cells. These have rather dense contents and prominent nuclei, and frequently have lignified walls, with pits which are particularly well-developed when they are paired with those present in the walls of adjacent vessels.

In the formation of tyloses, an increase in volume of the contents of xylem parenchyma cells, accompanied by the addition of new cell-wall material to the distended pit membranes, gives rise to balloon-like structures which protrude into the lumina of the vessels (Pl. 13, fig. 1). Very frequently the nuclei of the parent cells pass through into the tyloses, and starch grains may also accumulate in them. Occasionally, lignified reticulate thickening may form in the walls of the tyloses. A single xylem parenchyma cell may initiate several tyloses simultaneously, but usually the nucleus passes into one of them, and this one enlarges while the others remain small. Tyloses may become so numerous in a vessel that its lumen is completely occluded by them (Pl. 13, fig. 2).

Tyloses are uncommon in non-infected and undamaged plants, though they sometimes appear in vessels at the base of the stem at the end of the summer; they are frequently, though *not invariably*, found in plants infected with *V. albo-atrum*.

Mycelial growth and tylosis in relation to symptom intensity

The observations have shown that there is an inverse relationship between the intensity of tylosis and the intensity of mycelial growth within the vessels, and that the symptoms expressed by the infected plant consistently provide an indication of the relative importance of these two factors.

The acute syndrome. This corresponds with the expression of 'progressive' wilt caused by a virulent strain of *Verticillium* in a sensitive cultivar such as Fuggle. Symptoms are initiated at the base of the stem, and extend upwards: general chlorosis of the leaves is followed by marginal and interveinal necrosis which progresses towards the petioles and leads to the formation of abscission tissues and so to defoliation of the stem. Death of lateral shoots also occurs, and a brown discoloration of the whole xylem cylinder is evident from an early stage.

Preliminary observations which had drawn attention to the occurrence of tyloses in *Verticillium*-infected plants had suggested the possibility that, by causing extensive vascular obstruction, tyloses might induce

acute desiccation of the aerial parts of the plant and so give rise to the severe symptoms described above. This has not been supported by the extensive evidence which has since been obtained: in general it has been found that *in plants with very acute symptoms, the main vascular system is intensely colonized by hyaline mycelium, and tyloses are relatively few in number.*

Previous observations (Talboys, 1958) have indicated that, in young roots of a sensitive cultivar invaded by a virulent strain of the pathogen, the defensive reactions of the cortical and endodermal tissues fail to exclude the pathogen from the vascular system, with the result that the



Text-fig. 1. Diagram illustrating movement of a dye into the vascular system of a hop leaf with marginal and interveinal necrosis. The dye was present in veins shown by continuous lines, and was absent from those shown by dotted lines.

vessels of the infected root become invaded by masses of hyaline mycelium. It is now apparent that this type of invasion, once established, tends to extend into the main roots, into the stem (Pl. 13, fig. 3), into the petioles (Pl. 13, fig. 4), and into the main vascular bundles of the leaves. The fungus does not appear to enter the small terminal vascular elements in the interveinal areas of the leaf lamina.

By severing the stems of infected plants at the base and allowing the aerial parts to take up a solution of basic fuchsin and sodium metabisulphite (Talboys, 1955), it has been found that only a small proportion of the vessels in the stems and petioles are obstructed to such an extent that they are no longer available for transport of water. The dye passes readily into the leaves, even when they show clearly defined marginal and interveinal necrosis. Text-fig. 1 illustrates the distribution of the dye in the veins of a typical 'tiger-striped' leaf; it is apparent that the necrosis has not resulted from obstruction of the main vascular supply of the leaf.

It is interesting to note that occasionally conidia of *V. albo-atrum* have been found in association with mycelial masses in the vascular elements of plants showing advanced necrotic symptoms (Pl. 13, fig. 5), though there has been no direct evidence that dispersal of the pathogen within the host is accomplished by means of such conidia.

Though the typical 'progressive wilt' syndrome is normally associated

with infection of sensitive cultivars by highly pathogenic strains of *Verticillium*, acute symptoms can also arise in sensitive cultivars infected with a mild strain under conditions of high soil moisture, and even in tolerant cultivars such as Keyworth's Midseason and Whitbread's Golding Variety if they are planted in heavily infected soil where the nitrogen status is high. In all such circumstances, very acute symptoms are normally associated with profuse mycelial development and relatively slight tylosis.

The mild syndrome. This characteristically arises from infection of a sensitive hop cultivar by a mild strain of the pathogen, or of a tolerant cultivar by a highly pathogenic strain. General chlorosis and sectorial necrosis of the lower leaves leads to defoliation, which may slowly extend upwards for several feet. The lateral shoots arising from the axils of affected leaves, particularly in the upper parts of the infected region of the stem, frequently show no symptoms, and their profuse development may conceal the fact that partial defoliation of the primary axis has occurred. Some sectorial necrosis may occur in the upper parts of the plant, sometimes in a one-sided manner along the bine, but leaves with only small necrotic sectors are not shed until late in the season. Considerable thickening of the lower 1-2 ft. of the main stem usually occurs, and in transverse section it is found that an outer layer of unstained xylem surrounds a central cylinder showing the characteristic brown discoloration generally associated with vascular diseases of this type.

Numerous detailed observations have shown that almost invariably the development of mild symptoms is associated with very extensive tylosis of the vascular system in the roots and in the main stem.

Tyloses may be found in the roots and in the primary xylem and the first-formed secondary xylem of the lower part of the stem before any visible symptoms have developed. Subsequently their distribution becomes extended radially and longitudinally in the vascular cylinder of the stem, so that an increasing proportion of the xylem elements becomes blocked and therefore not available for water transport. However, the obstruction also appears to reduce very greatly the extent and intensity of colonization of the vascular system by the pathogen, and it is usually difficult to detect mycelium visually in sections of heavily tylosed vessels; even so, the fungus can usually be isolated from them by cultural methods, though the tyloses generally tend to be initiated in advance of the invading mycelium, sometimes by as much as one or two internodes. Direct colonization of tyloses or of xylem parenchyma cells has rarely been observed.

As in the acute syndrome, the status of vascular invasion of the plant as a whole seems to be referable to the situation which develops as a result of the host-parasite interactions in the extra-vascular tissues at sites of initial infections. It has been shown (Talboys, 1958) that cortical and endodermal defensive reactions in a tolerant cultivar appear to be capable of reducing, to a very low level, the invasion of the vascular system by the pathogen, and that even in a sensitive cultivar the mildly pathogenic strain of the fungus can be substantially excluded from the xylem; it has been noted that, under such conditions of limited vascular invasion, the occurrence of tyloses in the primary vascular elements of infected roots is fairly common.

The evidence as a whole suggests that the presence of small amounts of mycelium in the vascular system stimulates tylosis, whereas intense mycelial development prevents it. The observed relationships between symptom expression, mycelial development, and tylosis, can be satisfactorily interpreted if it is postulated that metabolites secreted by the pathogen in the vascular system of the host are, in respect of tylose formation, stimulatory at low concentrations but inhibitory at higher concentrations. This would account for the fact that the situation developed in the primary vascular elements of the infected root, as a result of the initial host-parasite interactions, appears to be self-perpetuating in the subsequent invasion of the main vascular system of the plant; intense initial colonization of the vessels would lead to suppression of tylosis, and therefore the way would be open for further intense colonization; very sparse initial invasion would stimulate tylosis, further extension of the fungus would be limited mechanically, and would therefore be accompanied by further tylosis.

It should be mentioned that, in experiments with *Verticillium* metabolites produced in liquid culture (Talboys, 1957), there was no evidence that the introduction of such substances into the vascular system of detached shoots led at all frequently to the formation of tyloses, though it is possible that the concentrations of metabolites used in these experiments fell within the inhibitory rather than the stimulatory range.

HYPERPLASIA OF THE XYLEM

As a result of the processes described above, a paradoxical situation arises in which plants with slight vascular obstruction show acute symptoms, while others in which a large proportion of the vessels are completely occluded by tyloses show only mild symptoms. The reason lies in the fact that extensive tylosis is almost always accompanied by renewed or abnormally prolonged cambial activity leading to the formation of additional secondary xylem. In this hyperplastic tissue, the vessels are all of small diameter, compared with the main vessels of the normal secondary xylem. Pl. 13, fig. 6, illustrates the condition usually found at the base of the stem of a plant showing characteristic mild symptoms: almost all of the vessels of the normal secondary wood have become completely obstructed by tyloses, but a broad ring of hyperplastic xylem has been formed. Vascular obstruction has also taken place in the older parts of the new tissue; only the relatively narrow outer ring of vascular tissues is available for water transport, and this is sufficient to maintain the life of the plant. However, the total cross-sectional area of this outer ring is only slightly less than that of the original secondary xylem. It may be noted that the potential water-conducting capacity of the normal secondary xylem cylinder is apparently considerably in excess of the general requirements of the plant; a transverse incision which severs half of the vascular cylinder of the stem causes no wilting or any other visible physiological disturbance to the plant as a whole.

With the formation of additional xylem the diameter of the stem is considerably increased, the epidermis and cortex break up, and an outer

cork layer develops. These processes result in the production of 'fat bine' or 'corky bine', which is a diagnostic feature of the mild syndrome, and in commercial hop gardens may be the only indication that the disease is present. It is a normal practice in such gardens to strip the leaves and laterals from the lower 3-4 ft. of the bine, and leaf symptoms which might have developed at that level are therefore not seen.

It has been mentioned that in infected plants which have not been 'stripped' some defoliation occurs, though the laterals arising from the axils of affected leaves may be undamaged. A partial explanation of this can be based upon the nature of the vascular supply to leaves and laterals (Pl. 14, fig. 7). The vascular tissues supplying the leaf arise in the primary xylem of the main stem and pass outwards to the petiole as a discrete bundle without forming secondary xylem; when the primary xylem of the stem becomes infected and tylosed, the leaf trace vessels also become obstructed, and even if the fungus does not enter the petiole the leaf will become desiccated. The conditions associated with tylose formation do not stimulate the development of additional xylem in the leaf trace as they do in the stem. The vascular supply to the lateral shoot also arises in the primary xylem, but secondary thickening takes place; the latest-formed wood of the lateral is continuous with the latest-formed wood of the main axis, and hyperplastic xylem formed in the main stem is also represented in laterals arising in the tylosed zone. A supply of water to the laterals is thus maintained. Tylosis in the laterals is irregular in distribution and, except in the lower ones, it generally causes little defoliation.

The formation of new xylem in association with vascular obstruction by tyloses is usually less evident in roots than it is in stems; there seems instead to be a tendency for new adventitious roots to develop on the underground parts of the stem.

When, under field conditions, infection is limited to a narrow sector of the vascular cylinder, there is usually no appreciable formation of hyperplastic xylem.

The mild syndrome does not always arise from the host-parasite combinations mentioned above; it is possible, for example, for a sensitive cultivar infected with a virulent strain of the pathogen to show mild symptoms if the nutritional status of the soil, particularly in respect of nitrogen, is very low.

Thus, considering the observations as a whole, it is clear that symptom intensity is not invariably associated with particular host-parasite combinations, but is associated with particular conditions of vascular invasion, which in turn appear to be determined by the effectiveness of the extra-vascular defensive reactions of the root. The many intermediate syndrome types which may be encountered in the field show corresponding variations from the extreme forms described above in the relative intensities of mycelial development, tylosis and hyperplastic xylem formation.

TYLOSIS AND XYLEM HYPERPLASIA INDUCED BY OTHER PATHOGENS

The formation of tyloses in the hop is not exclusively associated with invasion by *V. albo-atrum*; it may also be induced by *V. dahliae*, and by other, unrelated, organisms. It is well known that invasion of the base of

a hop bine by *Fusarium sambucinum* (hop canker) leads to a pronounced attenuation, the so-called 'growing off' symptom, so that the stem is readily pulled away from the main stock. Above the region of attenuation, the bine generally becomes greatly thickened and develops a corky appearance similar to the 'fat bine' condition in mild wilt. This has generally been attributed to accumulation of organic nutrients in an over-developed cortex as a result of the 'girdling' of the stem by the pathogen. This undoubtedly does occur, but an appreciable part of the enlargement is related to the formation of masses of tyloses in the vascular cylinder and to the associated development of hyperplastic xylem. This condition is not generally evident more than about 12 in. above soil level, and there is no brown discoloration of the xylem. It is not generally possible to isolate the pathogen from the xylem more than 1-2 in. above the attenuated zone, and it is rarely possible to see any mycelium in the woody tissues.

Obstruction of vessels in the roots and lower parts of the bines, by tyloses and mycelial masses, in a hop disease caused by a species of *Phytophthora* has been reported by Keyworth (1943). A transverse section of an infected stem, prepared by Keyworth, gives evidence suggesting that slight hyperplastic xylem formation may have occurred. It shows considerable mycelial development even in tylosed vessels, and also that invasion of the tyloses could take place.

TYLOSIS AND HYPERPLASIA AS WOUND-REACTIONS

The culture of hops involves training the plants on various types of wire-work and strings; in certain systems of stringing, notably the Butcher system (see Burgess, 1956), the bines are in contact with a horizontal wire about 4 ft. from the ground, and under windy conditions friction of the stem against this wire can sometimes lead to considerable local abrasion, which may extend inwards to the xylem. Mechanical damage of this kind invariably leads to the development of tyloses in the wood above and below the wound. They tend to occur in a similar manner in association with wounds arising from the normal practice of 'stripping' leaves and laterals from the lower parts of the plant, and in roots damaged by cultivation operations. In all these circumstances, the distribution of tyloses is usually closely limited to within a few inches above and below the lesion.

In gross lesions of this kind, where extensive callus tissue develops, the formation of new xylem elements probably occurs but is difficult to observe

Artificial induction of tyloses and hyperplastic xylem

Various attempts have been made to induce tyloses by injection of the vascular system with culture filtrates of the pathogen, but their formation has been infrequent and inconsistent. This may well reflect a subtle dosage requirement, which might be expected in view of the apparent influence of the quantity of mycelium in the vessels in determining the presence or absence of tyloses. It is also possible that an active metabolite might be secreted by the pathogen within the vascular system of the host but not in artificial culture.

The effects of various forms of artificial mechanical damage to the stem have been examined. It has been found that superficial abrasions or transverse incisions through the epidermis, cortex, phloem, and cambium cause no tylosis of the xylem, and no stimulation of xylem production, though, if such wounds are covered with moist cotton-wool and a polythene wrapping, adventitious roots are initiated within a few days.

Transverse incisions which enter the xylem invariably induce the formation of tyloses within 2–3 days in the vascular tissues immediately above and below the incision. In transverse sections taken in the vicinity of the wound, a wide sector of tylosed vessels is apparent, corresponding with the extent of the incision. Farther away from the wound, the sector of obstructed vessels becomes progressively narrower until it disappears completely and the whole vascular cylinder is again available for water transport; the effect of an incision at the centre of an internode is usually lost at, or a little beyond, the nodes which delimit the internode. Mention has been made of the fact that the potential water-conducting capacity of the stem exceeds the normal requirements; an incision putting 50–60 % of the vascular cylinder out of action causes no wilting or other leaf symptoms.

Tyloses can be induced by fine needle-punctures into the xylem, and in such instances only a single radial row of vessels may be damaged. In these vessels tyloses are formed within a few millimetres of the point of damage, and may only extend a few centimetres longitudinally. If such punctures are made with a hypodermic needle, and are injected with a spore suspension of *Verticillium*, tyloses are formed, but generally extend for several internodes above the point of injection. This is associated with a very sparse development of mycelium in the vessels. Small necrotic sectors may develop in some leaves; it is important to note that leaf symptoms very rarely arise from tylosis induced by mechanical damage alone.

When a *deep* transverse incision in a mature stem is kept moist, there is often a renewal of cambial activity above and below the wound, giving rise to a tissue closely resembling the hyperplastic xylem formed as a result of mild infections by *Verticillium* (Pl. 14, fig. 8). In the immediate vicinity of the wound the new xylem elements are orientated in such a way that they curve around the ends of it. It seems probable that eventually they would differentiate in callus tissues and cover the incision but this stage has not been observed. The association between tylosis and xylem hyperplasia following wounding appears to be similar to that in the 'fat-bine' condition of mild *Verticillium* wilt.

The nature of the stimulus that induces the renewal of cambial activity in such situations is not known. Attempts have been made to determine whether mere *obstruction* of the vessels will induce cambial re-activation or whether damage, e.g. to xylem parenchyma cells, is an essential prerequisite. So far, however, all efforts to inject dilute sodium pectinate solutions into the main stem through petioles or lateral shoots, with subsequent injection of calcium salts through other petioles to induce local calcium pectinate gel-formation in the vessels, have been unsuccessful. Premature obstruction takes place at the point of injection, and it seems probable that this is related to the acid reaction of the exposed tissues.

It is possible that, by selecting a pectinate of moderately high methoxyl content, and by careful buffering, this difficulty might be overcome. Similar experiments with alginates have also been unsuccessful.

HISTOLOGICAL FEATURES OF LEAF-SYMPTOM DEVELOPMENT

Reference has been made to the fact that, in the acute syndrome, development of marginal and interveinal necrosis of the leaves accompanies intense mycelial colonization of the vessels of the leaf trace, and that mechanical obstruction of the vessels does not appear to take place. In the mild syndrome sectorial necrosis of leaves is generally associated with the presence of tyloses in those vessels which would normally supply the necrotic regions, rather than with the presence of mycelial masses, though it seems probable that obstruction alone is not responsible for the death of the tissues; it has been found that cutting through one of the main vascular bundles at the base of a leaf causes no desiccation or any other visible damage, the isolated region apparently being supplied with water indirectly through the complex vascular reticulum of the lamina. Histological examination has suggested that the processes leading to necrosis are essentially similar in the two syndrome types.

Necrosis is invariably preceded by general chlorosis of the leaf tissues, and this is associated with degradation of the chloroplasts in the palisade and mesophyll layers. The chloroplasts may disappear completely, but the cells remain fully turgid and their nuclei appear to be normal (Pl. 14, fig. 9). The contents of the mesophyll cells adjacent to the terminal tracheidal elements of the vascular system then become modified, with the formation of brown granular masses which completely fill the cells (Pl. 14, fig. 10). Similar reactions occur, often a little later, in cells adjacent to the small vessels supplying the terminal tracheids. Subsequently, degeneration of the cell contents takes place in the mesophyll and palisade cells more remote from the terminal vascular elements; the appearance of the tissues suggests that dysfunction of the cells adjacent to the tracheids may lead to a failure of water transport to the more remote cells and hence to their desiccation. The walls of these cells turn brown, but usually, though not invariably, they remain intact. The contents also turn brown, and may either become shrunken, or may form amorphous resinous masses which fill the cells. There appears to be a tendency for cell-wall degeneration and intensive resin formation (Pl. 14, fig. 11) to be found more frequently in the sectorial type of necrotic region than in the interveinal 'tiger-stripe' type, where the general structure of the tissues tends to remain distinct and few cells other than those adjacent to the tracheids become filled with brown granular or resinous substances. However, these differences are not consistent.

It seems probable that, in both syndromes, the necrosis of leaf tissues results from the toxigenic action of the pathogen. The characteristic differences in the *distribution* of the necrotic tissues seem to reflect the degree to which the distribution of the pathogen within the vascular system is limited by the development of tyloses.

DISCUSSION

The observations described above suggest very strongly that the capacity of the host to respond to the presence of the pathogen in the vascular system by the formation of tyloses, together with the potentiality for a compensatory development of additional xylem, contribute significantly to the limitation of the pathogen and hence to the disease tolerance of the plant.

It is important to note that the *potentiality* for such reactions exists in the stems and roots of both sensitive and tolerant cultivars; their *expression* depends upon the presentation of a suitable stimulus, and this seems to be provided by the presence of a low incidence of mycelium in the vascular system. This condition is established when the cortical and endodermal defensive reactions are strongly developed and effectively retard the colonization of the vascular system (Talboys, 1958); it has been noted that, as a result of the apparent inverse relationship between tylosis and intensity of mycelial development, the conditions of vascular invasion which are *initially* established tend to be self-perpetuating in the subsequent colonization of the main vascular system.

Earlier observations (Talboys, 1957) had led to the suggestion that once certain specific conditions of invasion have arisen as a result of the host-parasite interactions in the root system, the ultimate fate of the individual plant is fully determined, even though at that stage symptoms may not have appeared above soil level. The period during which such conditions are established was defined as the 'determinative' phase of disease development; subsequent processes leading to symptom expression in the aerial parts of the plant would constitute an 'expressive' phase.

Anatomical studies of root invasion (Talboys, 1958) have indicated that the host-parasite interactions in the extra-vascular tissues of the root probably constitute an important part of the determinative phase, though it was pointed out that the limits of that phase could not be exactly defined. The observations described above now suggest that the determinative phase ends when the establishment of primary vascular invasion leads either to the stimulation or to the suppression of tyloses. Colonization of the main roots and of the aerial parts of the plant thus constitutes the expressive phase.

It has previously been pointed out (Talboys, 1958) that the defensive reactions of the extra-vascular tissues of the hop root are not uniquely associated either with the particular host or with the particular pathogen. The same comment may equally be applied to the occurrence of tyloses in the vascular system. Reference has been made to the fact that invasion of the hop by *Fusarium* and by *Phytophthora* can induce the formation of tyloses; their occurrence has similarly been reported by Struckmeyer, Beckman, Kuntz, & Riker (1954) in oak wilt (*Chalara quercina*), and by McClure (1950) in *Fusarium* wilt of sweet potato. Tyloses occur characteristically in many genera of plants (Eames & MacDaniels, 1947) and are usually associated with vascular dysfunction, e.g. they develop in the heartwood of many trees, and are frequently found in wounded vascular tissues. It is interesting to note that in some plants in which wounding

results in the obstruction of vessels by gums and resins, invasion by a vascular pathogen also induces the formation of these substances; an example of this is provided by the work of Brooks (1953) on the occlusion of *Stereum purpureum* by gum barriers in the plum. There appears to be some justification for regarding the occlusion of vascular pathogens by tyloses or gum deposits as expressions of general 'wound responses' by the host. The production of hyperplastic xylem in the hop may perhaps be regarded as an extension of this concept; the structural similarity of hyperplastic xylem and the 'wound-wood' formed in the vicinity of a transverse incision in the stem has been noted above. The proliferation of xylem following vascular invasion and tylosis does not seem to have been reported elsewhere.

The writer is greatly indebted to Miss D. P. Laurence for technical assistance, and to Miss J. M. Hay and Mr E. Y. Jones for the preparation of photomicrographs.

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EXPLANATION OF PLATES 13 AND 14

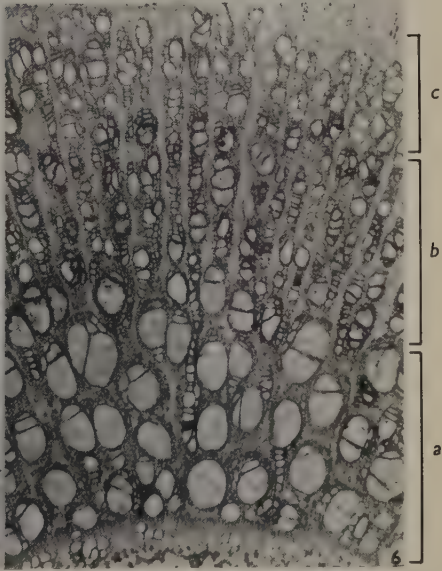
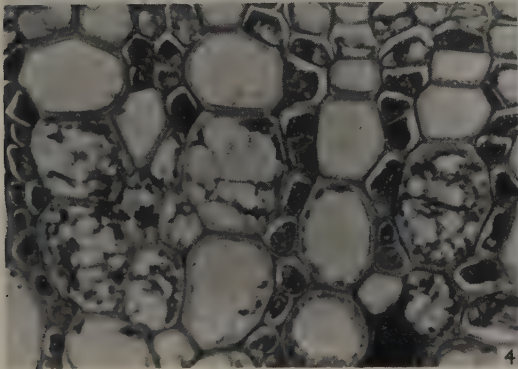
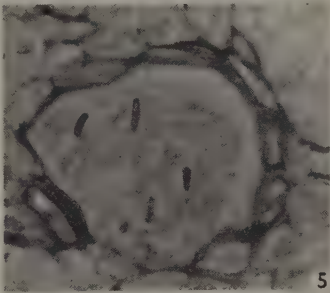
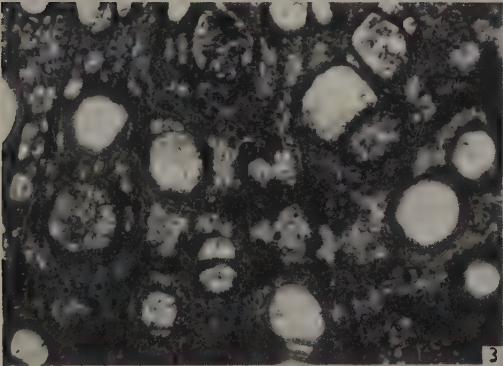
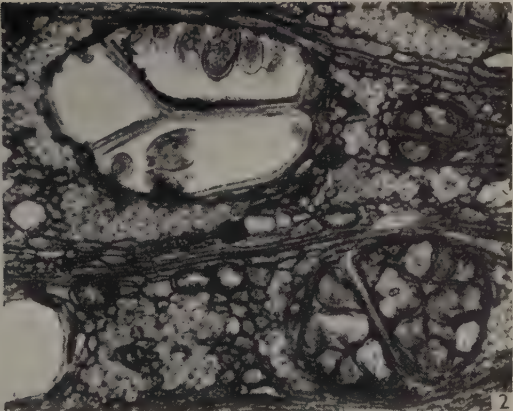
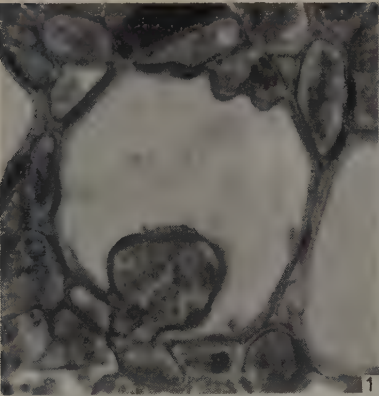
PLATE 13

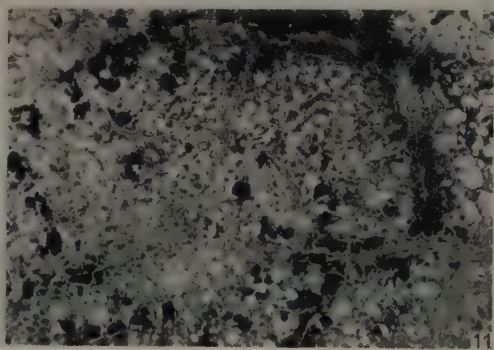
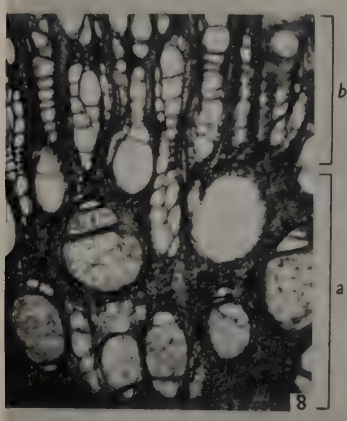
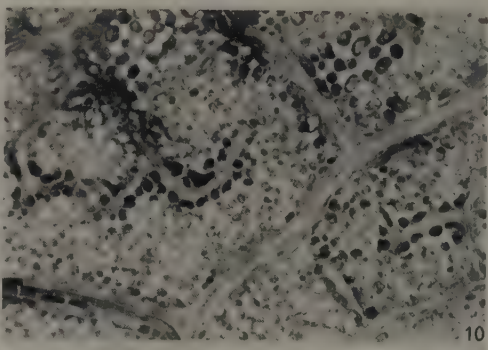
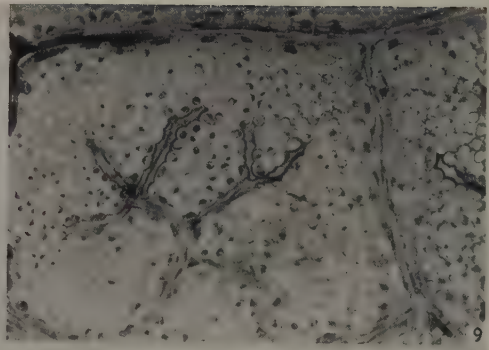
- Fig. 1. Early stages in development of tyloses from xylem parenchyma cells in a *Verticillium*-infected root of a tolerant cultivar (Transverse section).
- Fig. 2. Vessels partially and completely occluded by tyloses in a *Verticillium*-infected root of a tolerant cultivar. (T.S.).
- Fig. 3. Mycelial development in the stem of a sensitive cultivar infected with a virulent strain of *Verticillium* and showing acute symptoms (T.S.).
- Fig. 4. Mycelium in a leaf petiole of a sensitive cultivar infected with a virulent strain and showing acute interveinal necrosis (T.S.).
- Fig. 5. Mycelium and conidia of *V. albo-atrum* in a vessel of a root from a 'sensitive' plant showing advanced necrosis (T.S.).
- Fig. 6. The formation of 'fat bine' in a tolerant cultivar: (a) normal secondary xylem almost entirely obstructed by tyloses, (b) first-formed hyperplastic xylem also obstructed, (c) outer ring of hyperplastic xylem available for water transport (T.S.).

PLATE 14

- Fig. 7. A longitudinal tangential section through the outermost layer of hyperplastic xylem in a nodal region of a 'tolerant' plant showing mild symptoms: continuity of the secondary xylem of the axillary shoot (*a*) with the hyperplastic xylem of the main axis (*b*) is evident; the leaf-trace (*c*) has no connexion with the hyperplastic tissue.
- Fig. 8. Tylosis of secondary xylem (*a*) and the formation of 'wound-wood' (*b*) above a transverse incision in a stem (T.S.).
- Fig. 9. Terminal vascular elements in the chlorotic zone of a leaf which showed interveinal necrosis. (Section in plane of lamina).
- Fig. 10. Terminal vascular elements with surrounding deeply pigmented cells in an interveinal necrotic tissue.
- Fig. 11. Advanced degeneration and resin formation in a *sectorial* necrotic area from a plant with mild symptoms.

(Accepted for publication 27 September 1957)





BRITISH RECORDS, 21-24

21. **Scleroderris callunigena** (Karst.) Nannf. (Dennis in *Mycol. Pap. C.M.I.* No. 62, p. 6, 1956). Twice collected in August 1956 in Scotland on dead stems of *Calluna*, in each case in fine, mature, spore-bearing condition, (a) at Park, near Banchory, Aberdeenshire (Herb. W.D.G. 1113), and (b) Glen Cochill, near Dunkeld, Perthshire. Herb. W.D.G. 1114 and Herb. Kew. Asci $110-120 \times 8 \mu$, pore blue in Melzer reagent; spores $40-60 \times 2 \mu$, mostly 7-septate, arranged in the ascus in two slightly overlapping bundles of four.

W. D. GRADDON, *Congleton, Cheshire*

22. **Haglundia perelegans** (Hagl.) Nannf. (*N. Acta R. Soc. Sci. Ups.* p. 108, 1932). By the kindness of Dr R. W. G. Dennis I was privileged to see the first British material of this species found in October 1948 at Woburn Sands, Beds (*Bedfordshire Flora*, p. 470, 1953). Since then I have found it in two additional localities (a) in September 1951 at Moccas Park, Hereford—Herb. W.D.G. 581, and (b) in August 1957 at Maesmynis, Brecknockshire—Herb. W.D.G. 1207 and Herb. Kew. In all cases the fungus was growing on very rotten deciduous wood and it was scarcely possible to collect pieces carrying more than a few apothecia despite their growth in extensive colonies. The last gathering, on oak, was particularly fine, many of the specimens reaching 4 mm. diam.; the disk colour varied from slaty-blue to almost white and the exterior from pale to deep olive grey; young specimens are conspicuously softly hairy but this feature seems to diminish somewhat with age. The hymenium is characterized by very slender asci, $60-65 \times 3.5-4 \mu$ amidst abundant paraphyses; the spores measure $7-9 \times 1.5 \mu$.

W. D. GRADDON, *Congleton, Cheshire*

23. **Chytriomyces hyalinus** Karling (*Amer. J. Bot.* **32**, p. 363, 1945) was obtained from soil collected in Middlesex and Surrey, the Stour estuary, Dartmoor, and the Lake District. It grew only on chitin baits. Mature sporangia (Fig. 1b) were 12 to 50μ in diameter, and after dehiscence the operculum was seen only occasionally, lying free in the exit orifice (Fig. 1c) or attached to its rim. Zoospores were distinctly oval when in motion and $4.2-5 \mu$ long by $3-3.3 \mu$ wide with a hyaline oil globule $1.2-1.7 \mu$ in diameter and a posterior flagellum 20μ long (Fig. 1d). There was brief collective swarming of the zoospores before they dispersed.

(Very recently, these *Transactions*, **40**, p. 532, reported by F. K. Sparrow from Cavenham Heath and river Stour.)

L. G. WILLOUGHBY, *Freshwater Biological Association, Ambleside*

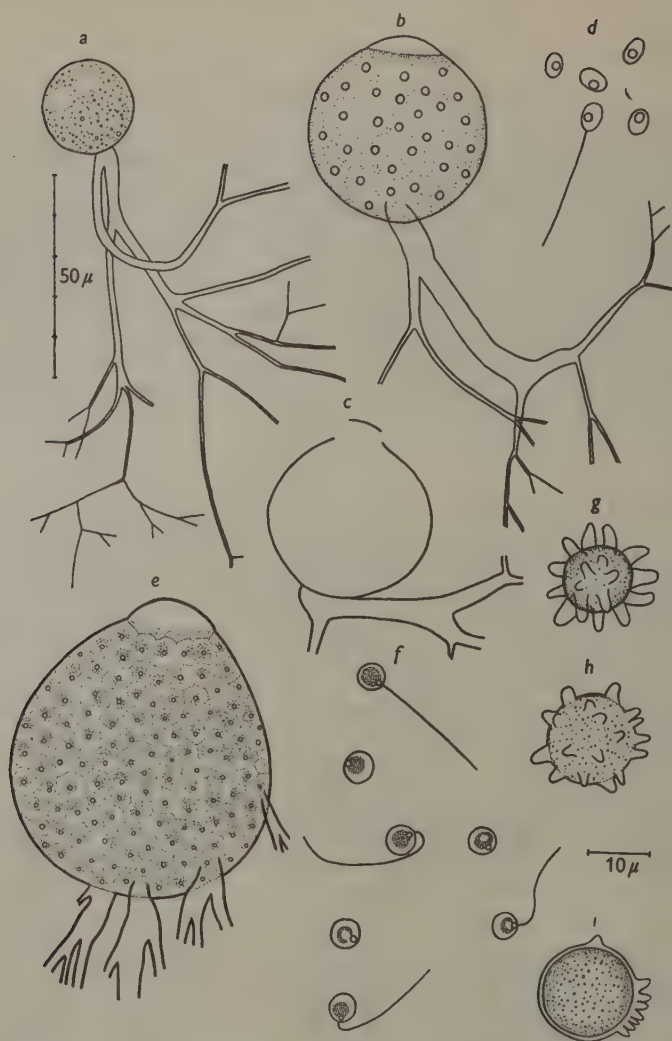


Fig. 1. *a-d*, *Chytridiomyces hyalinus* Karling. *e-i*, *Karlingia asterocysta* Karling. *a*, young thallus; *b*, mature sporangium; *c*, dehiscent sporangium; *d*, zoospores; *e*, mature sporangium; *f*, zoospores. *g-i*, resting spores; *i* is drawn in optical section. The scale to the left of *a* applies to *a* and *c* only.

24. ***Karlingia asterocysta*** Karling (*Mycologia*, **41**, p. 509, 1949) was also chitinophilic and was obtained from soil collected at Mistley, Essex. Mature sporangia were $16 \times 20 \mu$ to $93 \times 112 \mu$ and had a single exo-operculum (Fig. 1*e*) or more rarely two exo-opercula. Zoospores were spherical, $4.2-5 \mu$ in diameter, with a small hyaline oil globule situated near the flagellum insertion. The posterior flagellum was $15.5-24 \mu$ long (Fig. 1*f*). Resting spores were $12-25 \mu$ in over-all diameter, and ornamented with solid conical brown spines (Fig. 1*g-i*).

L. G. WILLOUGHBY, *Freshwater Biological Association, Ambleside*

PROCEEDINGS, 1957

MEETINGS

5 January 1957. Meeting held at the London School of Hygiene and Tropical Medicine, London, W.C. 1, at 11.0 a.m. Dr R. V. Harris, Chairman of the Plant Pathology Committee, in the Chair.

N. F. ROBERTSON. Observational evidence for some 'well-known' facts about blight.

J. M. HIRST. The early phases of potato blight epidemics.

J. GRAINGER. Host and parasite in potato blight.

P. M. AUSTIN BOMBE. The use of synoptic charts in forecasting potato blight.

E. C. LARGE. Progress in surveying and forecasting potato blight epidemics in England and Wales: 1950-1956.

A. E. COX. Potato blight control in North America.

See *Nature, Lond.*, **179**, 294-295, 1957, for an account of this symposium.

16 March 1957. Conversazione held in the Botany Department, Birkbeck College, London, W.C. 1, at 2.0 p.m. The President, Dr C. J. Hickman, in the Chair.

About forty demonstrations of mycological interest were displayed.

10 May 1957. Meeting at the Wellcome Museum of Medical Science, London, W.C. 1, at 2.0 p.m. The President, Dr C. J. Hickman, in the Chair.

The meeting took the form of visits to a new series of exhibits of human mycoses, prepared by the Museum with the help of J. E. Mackinnon and G. C. Ainsworth, and to a selection of early literature on medical and veterinary mycology in the Wellcome Historical Medical Library. A film on Coccidioidomycosis made by Dr L. Ajello was also shown. Members were entertained to tea by the Wellcome Foundation, and in conclusion the President thanked the Director of the Museum, Col. S. A. Bozman, and his staff for their hospitality.

11 October 1957. Meeting held at Birkbeck College, London, W.C. 1, at 11.0 a.m. The President, Dr. C. J. Hickman, in the Chair.

K. B. RAPER. Nomenclature in *Aspergillus* and *Penicillium*.

See *Mycologia*, **49**, 644-662, 1957.

N. F. ROBERTSON. *The water relations of hyphal apices*. See *Ann. Bot., Lond.* (in the Press).

L. S. LLOYD. The perithecial development of *Lulworthia medusa*.

The perithecia of *Lulworthia medusa* (Ellis & Everhart) Cribb & Cribb, a saprophytic marine Pyrenomycete, occur in the air chambers of *Spartina townsendii*.

A coiled ascogonium, consisting of multinucleate segments, first appears invested in a spherical plexus of hyphae which form the wall of the perithecial (ascocarp) initial. Both sterile and fertile perithecial centrum tissues originate from the ascogonium. A

sterile pseudoparenchymatous centrum of large, inflated cells occupies the perithecial cavity and is gradually invaded and replaced by the expansion of the fertile ascogenous tissue. Binucleate ascogenous cells are formed from multinucleate cells of the fertile centrum and in turn croziers are formed to give rise to binucleate asci. Crozier proliferation by the fusion of uninucleate stalk and terminal cells occurs, resulting in the production of asci of different age in the fertile centrum. The perithecial neck originates from the inner perithecial wall and an ostiolar canal is formed mainly schizogenously, but partly lysigenously at the base. Periphyses line the canal.

Nuclear fusion in the young ascus is followed by an apparent increase in length of the bivalents during prophase. Meiosis and an ensuing mitosis result in the formation of eight nuclei. Subsequently, eight long filiform ascospores, with the typical hyaline apiculi of the genus *Lulworthia*, are delimited within the unitunicate ascus membrane. During nuclear divisions in the ascus two spherical bodies (centrospheres) appear in the cytoplasm and become associated with the poles of each nucleus before the formation of crescentic intra-nuclear centrosomes. Five bivalents are seen during prophase of the first ascus division and five haploid chromosomes during subsequent stages of nuclear division in the ascus.

Ascospores are released in a bundle into the perithecium after deliquescence of the uniformly thin cylindro-clavate ascus membrane and are discharged singly, but continuously, on drying of the ascocarp tissues. Ascospores germinate and grow on sea-water agar, and mature ascocarps are formed after 6-9 months on *Spartina* stems immersed in agar slants.

J. A. PATEMAN. Variation in ascospore size in *Neurospora crassa*.

See *Heredity* (in the Press).

ANNUAL GENERAL MEETING

Saturday, 7 December 1957. The sixty-first Annual General Meeting was held in the rooms of the Linnean Society of London at 12.0 noon with the President, Dr C. J. Hickman, in the Chair.

After the Minutes of the previous Annual General Meeting had been read and signed, the President recorded with regret the deaths during the year of Mrs A. Bacon, Mr F. H. Brokenshire, Mr H. J. Howard and Dr C. L. Shear. He then briefly reviewed the year's activities.

The Treasurer presented his Annual Statement which was accepted *nem. con.*

The following Officers and Council were elected for 1958: *President*: F. B. Hora; *Vice-President*: Dr Mary Glynne; *Treasurer*: W. Buddin; *Secretary*: J. G. Manners; *Foray Secretary*: Mrs S. M. Francis; *Plant Pathology Committee Secretary*: E. W. Buxton; *Editors*: G. C. Ainsworth and S. D. Garrett; *Members of Council*: J. L. Harley, J. C. Hopkins, and J. Webster (retiring members: Dr A. H. S. Brown, D. A. Reid, J. T. Savory); *Members of Foray Committee*: Miss J. Bywater and C. Booth; *Members of Plant Pathology Committee*: J. J. Baker, J. Rishbeth and P. W. Talboys.

At 2.30 p.m. the President gave an address entitled 'Phytophthora—Plant Destroyer'. A vote of thanks to the President for his address was moved by Miss E. M. Blackwell, seconded by Prof. P. H. Gregory, and carried with acclamation.

THE THIRTY-FIRST ANNUAL PLANT PATHOLOGY
FIELD MEETING

4-5 JULY 1957

This very successful meeting was held in the Edinburgh district. Of the 50 members who attended, many stayed in Masson Hall, one of the hostels of Edinburgh University. The first visit on the morning of 4 July was to the Plant Breeding Station, Pentland field. In welcoming the party, the Director, Dr J. W. Gregor, offered a choice between visits to field plots and glasshouses or an indoor programme. Despite inclement conditions underfoot members decided on the former and were shown interesting experiments in progress on potato virus diseases and breeding for resistance to potato blight. After lunch at Colinton, we visited the headquarters of the Scientific Services of the Department of Agriculture for Scotland by permission of Dr T. P. McIntosh; there we examined more plots and many interesting indoor demonstrations. Dr Mary Noble took us to a field where there were wheat plants infected with *Cephalosporium* sp.; not to be out-done the visitors drew attention to oat plants with symptoms suggesting infection with cereal yellow dwarf virus. In the evening members were the guests of the Botanical Society of Edinburgh at the Royal Botanic Garden, where in addition to further demonstrations we were treated to a conducted tour of this beautiful garden and refreshments.

Next day we visited nurseries in the Clyde Valley, seeing, and tasting, a wide variety of horticultural crops. First, at Garrison Bridge Farm (Mr J. Warnock) where, although there were diseases to interest us, the production was obviously intensive and successful. While with Mr D. Carmichel of Carluke we were impressed by the strawberries he had bred, by the way in which he had countered 'red-core' and not a little amused by the forthright way in which he gave his opinions and answered our questions. We were grateful to all the local organizers, particularly Dr Noble and Dr C. E. Foister, for all they had done and not least because they allowed us to share some of their unsolved problems, of glasshouse design at Overton Farm, Crossford (Prof. J. Kirkwood) and of plum canker at Underbank Nurseries, Crossford, where Mr and Mrs A. D. Crouch provided us with a generous and most welcome tea.

J. M. HIRST

Secretary, Plant Pathology Committee

THE PORLOCK FORAY

22-26 MAY 1957

By S. M. FRANCIS, *Foray Secretary*

The twenty-eighth Spring Foray was held at Porlock in Somerset from 22 to 26 May. About twenty members stayed at the Castle Hotel where the proprietors, Mr and Mrs Billcliffe, did all they could to make us really comfortable. A fine workroom had been discovered by Miss Waterhouse; this was the Methodist Hall which we were allowed to use by courtesy of the Minister.

On Thursday, 23 May, we visited Selworthy, working up the Combe, over the hill and down the far side to Bossington. Friday was spent at Dulverton in Burrige Woods on the west bank of the River Barle. We are grateful for permission to collect in these woods from the owners Mrs Turner and Col. Clayton. After lunch we drove to Tarr Steps and members worked (or walked) through the woods on either side of the river. The weather was fine and warm and an ice-cream van waiting at the Steps did a profitable trade on our return. Horner Woods, to the south of Porlock, were searched on Saturday, some members spending the whole day in the area while others made a shorter visit.

On Sunday morning most of us took advantage of an offer by Mr Norman Hadden (a former member of the Society) to show us his famous gardens. We spent a most pleasant morning looking and marvelling at all the treasures that Mr Hadden has managed to pack into his gardens.

There were three comments on the 'List of Species'. Mr Orton collected what is probably a new species of *Mycena* on alder debris, which has been found once before in East Devon. Mr Booth reports a new *Pyrenomycete* record, a collection made at Selworthy of *Thyridaria minima* (Ell. & Ev.) Wehmeyer. The list also includes seven other *Pyrenomycetes* which are rarely collected. *Sebacina laccata* Bourd. & Galz. collected by Dr Parker-Rhodes is also a new record and has been deposited in the herbarium at Kew.

I am once again most grateful to members for the help they have given me in compiling the Species List, in particular: Dr A. H. S. Brown, Dr L. E. Hawker, Miss Waterhouse, and Messrs Austwick, Booth, M. B. Ellis, Graddon, Orton, Parker-Rhodes, and Reid. I would also like to thank the members who set to so willingly to transform the Methodist Hall into such a superior workroom and who did not forget to turn it back into a Methodist Hall.

List of species

A. = Ashley Combe; B. = Bossington; D. = Dulverton; H. = Horner Wood; P. = Porlock; S. = Selworthy; T. = Tarr Steps. † = a new British record.

Author citations as in British lists, for full details see these *Transactions* 38, 185 (1955).

NEW RECORDS

- (1) *Thyridaria minima* (Ell. & Ev.) Wehm., on *Acer*, S.; deposited at the Commonwealth Mycological Institute as I.M.I. 69497. (Mr C. Booth.)
- (2) *Sebacina laccata* Bourd. & Galz., on *Fraxinus*, P.; deposited in the herbarium, Kew. (Dr Parker-Rhodes and Mr D. Reid.)
- (3) *Poria ferrea* (Pers.) Overholts, B. and Cloutsham Valley; deposited in the herbarium, Kew. (Mr D. Reid.)

MYXOMYCETES

Arcyria nutans, on wood, H.
Badhamia utricularis, on wood, D.
Comatricha typhoides, on wood, H.
Fuligo septica, H.
Hemitrichia vesparium, on wood, D.
Lycogala epidendrum, on wood, H., P.
Reticularia lycoperdon, on wood, D.
Stemonitis splendens, on wood, H.
Trichia botrytis, on sawdust and wood chips, P.; *persimilis*, on wood, H.

PHYCOMYCETES

Bremia lactucae Regel, on *Taraxacum officinale*, P.
Entomophthora aphidis Hoffm., B.
Peronospora alta Fckl., on *Plantago major*, P.; *aparines* (de Bary) Gäum., on *Galium aparine*, S.; *effusa* (Grev.) Rabenh., on *Chenopodium* sp., P.; *ficariae* Tul., on *Ranunculus ficaria*, D.; *grisea* Unger, on *Veronica beccabunga*, H., *V. hederifolia*, H., *V. ?persica*, P.; *thlaspeos-arvensis* Gäum., on *Thlaspi arvense*, P.; *valerianae* Trail, on *Valeriana officinalis*, D.; *viciae* (Berk.) Casp., on *Vicia sepium*, P.
Plasmopara densa (Rabenh.) Schroet., on *Rhinanthus* sp., D.
Pythium undulatum Petersen, on bud scales of *Acer pseudoplatanus* in water, D.

HEMI-ASCOMYCETES

Protomyces inundatus Dangeard, on *Apium* sp., B.
Taphrina deformans (Berk.) Tul., on *Prunus amygdalus*, P.; *tosquetii* (West.) Magnus, on *Alnus*, A.

EU-ASCOMYCETES

PYRENOMYCETES

Bertia moriformis, on wood, T.
Calosphaeria parasitica Fckl., on *Fagus*, S.
Calyculosphaeria tristis, on *Fagus*, S.
Ceratostomella ampullasca, on *Alnus*, H.; *lejoscarpa* Sacc., on wood, T.
Chaetosphaeria innumera, on *Alnus*, H.; *myriocarpa* (Fr.) Booth, on *Quercus ilex*, B.; *phaeostroma*, on *Fagus*, S.; *pulviscula* (Currey) Booth, on *Ulmus*, D.
Clypeosphaeria notarisii, on *Rubus*, P.
Cordyceps forquignoni, on *Musca* sp., B.; *militaris*, on insect larva, H.
Coronophora angustata, on *Sorbus*, S.
Cryptosphaeria eunomia, on *Fraxinus*, S.
Diaporthe eres, on *Fraxinus*, S.; *padi* Oth., on *Sorbus*, S.
Diatrypella favacea, on *Betula*, S.
Erysiphe cichoracearum, on *Plantago major*, P.
Farlowiella carmichaeliana, on *Corylus*, T.
Gloniopsis levantica Rehm, on *Quercus ilex*, L.
Hypocrea citrina, on *Polyporus betulinus*, on *Betula*, H.
Hypomyces aurantius, on agaric, B.; *rosellus*, on *Polystictus versicolor*, S.
Hypoxylon fuscum, on *Corylus*, T.; *fragiformis* (Pers.) Kick., on *Fagus*, S.; *howeanum*, on *Acer*, S.; *semi-immersum*, on *Quercus ilex*, S.; *serpens*, on *Quercus ilex*, B.; *rubiginosum*, on *Fraxinus*, S.

- Hysterium angustatum*, on *Fraxinus*, *S.*
Lasiosphaeria caudata (Fckl.) Sacc., on *Crataegus*, *T.*; *spermoides*, on wood, *T.*; *strigosa*, on wood, *T.*
Melanconis stilbostoma, on *Betula*, *S.*
Nectria flavo-viridis (Fckl.) Wollenw., on *Corylus*, *T.*; *sanguinea* (Bolt.) Fr., on *Fagus*, *S.*
Ohleria rugulosa Fckl., on *Alnus*, *H.*
Ophiobolus acuminatus, on *Cirsium vulgare*, *S.*; *rubellus*, on Umbellifer stem, *P.*
Ophionectria cerea (Berk. & Curt.) Ell. & Ev., on *Fraxinus*, *S.*
Physalospora mutila, on *Fraxinus*, *S.*
Pleomassaria siparia, on *Betula*, *S.*
Polystigma rubrum, on *Prunus spinosa*, *P.*
Pseudovalsa lanciformis, on *Betula*, *S.*
Rosellinia aquila, on wood, *T.*
Sillia ferruginea, on *Corylus*, *D.*
† *Thyridaria minima* (Ell. & Ev.) Wehm., on *Acer*, *S.*
Tympanopsis euomphala, on *Fraxinus*, *S.*
Zignoella collabens, on wood, *T.*

DISCOMYCETES

- Aleuria sepiatra* (Cooke) Boud., *S.*
Apostemidium fiscellum Karst., *S.*
Dasyscypha cerina (Pers.) Fckl., on *Quercus ilex*, *S.*; *fuscescens* (Pers.) Rehm, *D.*; *leucophaea* (Pers.) Mass., *Dunster.*
Eriopeziza caesia (Pers.) Rehm, *T.*
Helotium clavus (A. & S.) Gill., *H.*, *S.*, *T.*; *clavus v. grandis* (Boud.) Dennis, *S.*; *imberbe* (Bull.) Fr., *S.*; *repandum* Philips, *Cloutsham Valley.*
Hyaloscypha dematiicola (Berk. & Br.) Nannf., on *Quercus*, *S.*; *hyalina* (Pers.) Boud., *H.*; *stevensoni* (Berk. & Br.) Nannf., *D.*
Mitrla paludosa, Fr., *S.*
Orbilina inflatula Karst., *H.*; *xanthostigma* Fr., *H.*
Pachyella babingtonii (Berk. & Br.) Boud., *H.*
Phialea cyathoidea (Bull.) Gill., *H.*; *pteridicola* (Crouan) Gill, *H.*
Propolis faginea (Schr.) Karst., on *Prunus spinosa*, *H.*
Pyrenopeziza lychnidis (Desm.) Rehm, on *Melandrium rubrum*, *D.*, *H.*; *mercurialis* (Fckl.) Boud., *T.*
Scutellinia scutellata (Linn.) Lamb, *H.*, *S.*
Spilopodia melanogramma Boud., on *Mercurialis perennis*, *T.*
Tapesia fusca (Pers.) Fckl., *H.*
Vibrissea truncorum Fr., *T.*

HETERO-BASIDIOMYCETES

TREMELLALES

- Auricularia auricula-judae*, on *Sambucus*, *S.*; *mesenterica*, on *Juglans*, *B.*
Dacrymyces stillatus, on *Pinus*, *B.*; on *Hedera*, *S.*
Exidia glandulosa, on *Ilex*, *B.*; *nucleata*, on *Fagus*, *S.*
Sebacina epigea var. *goniophora* Bourd. & Galz., on *Fraxinus*, *D.*; *fugacissima* f. *sebacea*, on *Quercus*, *T.*; † *laccata* Bourd. & Galz., on *Fraxinus*, *P.*
Tremella foliacea, on *Corylus*, *D.*; *mesenterica*, on *Quercus*, *T.*

UREDINALES

- Melampsora hypericorum*, on *Hypericum androsaemum*, *P.*
Melampsorella caryophyllacearum, on *Stellaria holostea*, *H.*
Milesia kriegieriana, on *Dryopteris austriaca*, *D.*; *scolopendrii*, on *Phyllitis scolopendrium*, *H.*
Phragmidium fragariastrum, II, on *Potentilla sterilis*, *D.*; *violaceum*, I, on *Rubus* sp., *D.*, *S.*
Puccinia adoxae, III, on *Adoxa moschatellina*, *H.*; *annularis*, on *Teucrium scorodonia*, *D.*; *arenariae*, on *Melandrium rubrum*, *D.*; *Moehringia trinerva*, *S.*; *chrysosplenii*, III, on *Chrysosplenium* sp., *T.*; *conii*, II, on *Conium maculatum*, *B.*; *epilobii*, III, on *Epilobium* sp., *D.*, *P.*; *obtegens* on *Cirsium arvense*, *S.*; *primulae*, I, III, on *Primula vulgaris*, *D.*, *P.*, *T.*; *sessilis*, I, on *Arum maculatum*, *H.*; *smyrnii*, I, III, on *Smyrnum olusatrum*, *H.*, *P.*; *veronicae*, III, on *Veronica montana*, *B.*, *D.*, *S.*, *T.*

Uromyces betae, III, on *Beta vulgaris* subsp. *maritima*, P.; *scillarum*, on *Endymion non-scriptus*, D.; *valerianae*, on *Valeriana officinalis*, D.

USTILAGINALES

Ustiloma helosciadii, on *Oenanthe crocata*, T.

Ustilago violacea, on *Silene maritima*, P, *Melandrium rubrum*, D., S., T.

HOMO-BASIDIOMYCETES—HYMENOMYCETES

APHYLLOPHORALES

Acia uda, S.

Corticium amianthinum Bourd. & Galz., on wood, P.; *centrifugum* (Lév.) Bres., on wood, B.; *filium* Bres., on *Quercus*, H.; *submutabile* v. Höhn. & Litsch, on moss on *Fraxinus*, H.; on *Corylus*, T.; *suecicum* Litsch apud Lund & Nannf., on *Fraxinus*, D.

Daedalea unicolor (Bull.) Fr., on *Quercus*, D.

Exobasidium vaccinii, B.

Fomes ferruginosus, on *Quercus*, T.; *fraxineus*, on *Juglans*, B.

Ganoderma applanatum, on *Fagus*, S.

Gloeocystidium lactescens, on *Betula*, S.; *pallidum*, on *Ilex*, B.; *porosum*, on *Corylus*, D.; *tenue*, on rotten wood, S.

Grandinia granulosa, S.; *microspora* Karst., on rootten wood, H.; on *Pinus*, B.; on rotten wood, H.

Hydnum cyathiforme, S.

Hymenochaete rubiginosa, on *Quercus*, D.; *rubiginosa* subsp. *subfuliginosa* Bourd. & Galz., on *Corylus*, D., on *Quercus*, P.

Peniophora aegerita (Aegerita stage), P.; *byssoides*, on *Quercus*, H.; *gigantea*, on *Fraxinus*, P.; *hydroides*, on *Fraxinus*, D.; *incarnata* subsp. *versicolor* Bres., on *Hedera*, S.; *longispora*, on *Fraxinus*, H.; *nuda*, on *Quercus*, H., on *Acer*, S.; *pallidula*, on *Picea*, B.; *pubera*, on *Quercus*, H.; *roumeguerii*, on *Quercus*, P.

Polyporus adustus, H.; *betulinus*, S.; *brumalis*, on *Betula*, S.; *dryadeus*, H., S.; *radiatus*, H.; *reticulatus*, on *Fraxinus*, H.; *semipileatus*, on *Quercus*, H., P.; *sulphureus*, on *Quercus*, D.

Polystictus abietinus, on *Pinus*, S.; *versicolor*, D., H., S., T.

†*Poria ferrea* (Pers.) Overholts (= *Phellinus ferreus* (Pers.) Bourd. & Galz.), B. and Cloutsham Valley; *reticulata*, on *Fraxinus*, H.; *vaillantii*, on *Ilex*, B., *versipora* (Pers.) Baxter, S.

Stereum hirsutum, D., H., S., T.; *rugosum*, on *Alnus*, T.

Trametes mollis, T.; *serpens*, on *Corylus*, T.; *sinuosa* (= *Poria sinuosa* Fr.), on *Betula*, S.

AGARICALES

Amanita rubescens, S.

Boletus erythropus, S.

Coprinus domesticus sensu Kühner & Romagnesi, B.; *hemerobius* sensu Lange, H.; *lagopus*, S.; *micaceus*, S.

Crepidotus luteolus, B.; *pubescens* sensu Kühner & Romagnesi, H.

Hypholoma fasciculare, S.

Galera nana, D.

Marasmius dryophilus, S.; *tenacellus*, B.

Mycena alcalina, D.; *ammoniaca*, D.; *subalpina*, S.

Naucoria carpophila, A.; *granulosa*, S.; *semiorbicularis*, S.

Omphalia umbellifera, T.

Panus stipticus, B.

Pleurotus ostreatus, B.

Pluteus cervinus, D., H.; *umbrosus*, B.; *petasatus*, B.

Psathyrella disseminata, B.; *fusca* (Schum. ex Lange) Moser, S.; *obtusata*, S.

Schizophyllum commune, T.

Stropharia semiglobata, S.

Tubaria furfuracea, S.

HOMO-BASIDIOMYCETES—GASTEROMYCETES

Bovista nigrescens, S.

FUNGI HYPOGEI

PHYCOMYCETES

Endogone lactiflua, S.

ASCOMYCETES

Elaphomyces muricatus, T.

BASIDIOMYCETES

Hydnотrya tulasnei, S.

Hysterangium thwaitesii, S.

Melanogaster ambiguus, S.

FUNGI IMPERFECTI

COELOMYCETES

Cytospora rubescens, on *Sorbus*, S.

Diplodia pinea, on *Pinus*, S.; sarmentorum, on *Acer*, S.

HYPHOMYCETES

Acladium conspersum Link, on dead wood, T.

Aegerita stage—see *Peniophora aegerita*.

Bispora pusilla Sacc., on *Ilex*, D.

Brachysporium apicale (Berk. & Br.) Sacc., on *Quercus*, T.; *bloxami* (Cooke) Sacc.; on rotten wood, D., S.; *obovatum* (Berk.) Sacc., on *Fagus*, D., S.

Cercospora mercurialis Pass., on *Mercurialis perennis*, H.

Gonytrichum macrocladum (Sacc.) Hughes, on rotten wood, T.

Graphium calicioides (Fr.) Cooke & Masee, on *Quercus*, S.

Haplaria grisea Chev., on *Acer*, S.

Helminthosporium simplex, Fr., on wood, H.; *velutinum* Fr., on *Alnus glutinosa*, H.

Menispora ciliata Corda, on *Betula*, T.

Monotospora sphaerocephala Berk. & Br., on *Quercus*, S.

Oidium aureum Fr., on *Betula* and *Fagus*, S.

Ovularia primulana Karst., A.

Paecilomyces elegans (Corda) Mason & Hughes, on *Pinus*, B.; *farinosus* (Fr.) Brown & Smith, on an insect, S.

Periconia cambrensis Mason & M.B.Ellis, on rotten wood, D.

Ramularia lampsanae (Desm.) Sacc., H.; *variabilis* Fckl., on *Digitalis purpurea*, H.

Septonema pallidum (Grove) Hughes, on rotten wood, T.

Speira toruloides Corda, on *Alnus*, H.

Sporoschisma juvenile Boud., on *Ulmus*, S.; *mirabile* Berk. & Br., on wood, T.

Stilbum tomentosum Fr. var. *ovalispora* A.L.Smith, on *Trichia* sp., D.

Torula ramosa Fckl., on rotten wood, D.

Trichoderma viride Fr., on *Fagus*, S.

Tripodosporium elegans Corda, on *Quercus*, S.

Verticillium cyclosporum (Grove) Mason & Hughes, on *Quercus*, B., T.; *sulphurellum* Sacc., on *Quercus*, S., T.

Xylohypha nigrescens (Fr.) Mason, on *Fraxinus*, S.

REVIEWS

Plant Diseases due to Bacteria. By W. J. DOWSON. (Cambridge University Press, 1957.) Pp. xv + 232, 30 figures, 21 maps. Price: 32s. 6d.

The publication in 1949 of the original edition of this book under the title of *Manual of Bacterial Plant Diseases* was something of a landmark in the literature of plant pathology, for nothing comparable had appeared since Erwin Smith's *Introduction* in 1920. The recent appearance of a revised edition of Sorauer's *Handbuch* has not materially affected the situation, for its treatment of the subject is quite different and Dr Dowson's book still remains the only up-to-date account of the bacterial diseases of plants in the English language.

This new edition follows closely the pattern of the old, except for the addition of a chapter on the preservation of cultures. There are three sections, the first dealing with general matters, e.g. classification and nomenclature, the second with media and staining techniques, and the third and largest section with the diseases themselves arranged under the names of individual pathogens. Much of the material has been rewritten or amplified in the light of new information, and more recent references added. Several new illustrations have been used and the standard of production of these is much improved.

As a systematist Dr Dowson is frankly controversial. Originally all peritrichous bacteria were grouped under *Bacterium*, but this was subsequently invalidated as a generic name by the International Committee on Bacterial Nomenclature (1953). As a result Bergey's system, with its two peritrichous genera *Erwinia* and *Agrobacterium*, came into common usage in this country, although it had long been established in the U.S.A. and elsewhere. Dr Dowson now proposes *Erwinia* for all peritrichous forms, with the exception of the soft-rot organisms which he separates into *Pectobacterium* on the basis of their ability to liquify pectate gel.

This is no place to discuss the rival merits of the two systems. The differences between them affect comparatively few organisms, but the differences exist and will undoubtedly add to the existing confusion. It is clear now, if it were never clear before, that we are still a long way from a stable system of nomenclature, and that present difficulties are unlikely to be resolved until further work has been done on the natural affinities of these organisms. This applies equally to the problem of differentiating species within the genera for this is by no means as straightforward as is sometimes suggested.

It is quite unnecessary to stress the value of this book, for this should be manifestly obvious. Its simple yet comprehensive treatment of the subject is exceptional and comes as something of a relief from those tedious compendiums which tend to be regarded as the hall-mark of authority. Since its original publication in 1949 no plant pathologist can have had any excuse for professing ignorance of the plant pathogenic bacteria or the diseases they cause.

J. E. CROSSE

Ustilaginalele din Republica Populară Română. By TRAIAN SĂVULESCU. (Academiei Republicii Populare Romine. 1957.) 2 vols. pp. 1-(544), (545)-1168, 20 col. plates, 594 text-figures. Price not stated.

These two large and lavishly produced quarto volumes on the smuts are a sequel to the author's monograph on the Romanian rusts already reviewed in these *Transactions* (37, p. 178, 1954). The first volume comprises a general account of the morphology, biology distribution, pathogenicity, toxicity, and control of the Ustilaginales with special reference to the smuts of Romania to the knowledge of which the author has made so many contributions during the past thirty years. The second includes keys to, and illustrated descriptions of, the two hundred and eight species recorded for Romania.

The taxonomic treatment is conservative. The modern trend to consolidate morphologically similar smuts on related hosts is not followed and it is a pity that the names adopted for the pathogens of such important diseases as maize smut and classical wheat bunt do not conform to the International Code.

There are numerous tabulations in the general part, all sections are fully documented, and the two volumes will find a place alongside the monographs of Liro and Ciferri where they will frequently be consulted by students of European smuts.

G. C. AINSWORTH

The Friendly Fungi. A New Approach to the Eelworm Problem. By C. L. DUDINGTON. (Faber & Faber, 1956.) Pp. 188, 24 plates, 7 text-figures. Price: 21s.

Much of the recent increase in our knowledge of predacious fungi and their activities can be attributed to the work of Dr Duddington on this interesting group. He is one of the leading authorities on these fungi and this book is a most welcome and necessary addition to mycological literature. It is written in a style which is stimulating and often amusing. It is informative and yet is written, as far as possible, in non-technical language which can be readily understood and appreciated by those whose interest exceeds their specialized knowledge.

The book covers every aspect of our knowledge of this fascinating group of fungi and much of it is devoted to a description of the various mechanisms employed for the capture of the eelworms on which they prey, and the methods by which they can be studied. This section is admirable and is accompanied by many excellent photographic plates and line drawings. The rest of the book is more controversial, dealing as it does with experiments on and prospects for the use of predacious fungi in the biological control of nematode pests. Here, even the title pre-judges the issue, a tendency to which Dr Duddington succumbs on several occasions throughout this section. In the reviewer's opinion, some of the experiments described would have been more convincing had they been based on estimations of the eelworm populations in the soil before and after treatments, and on the level of invasion of the host crop. The crop yield, which in the main has been used as the criterion, is affected by a variety of factors and is therefore not an entirely reliable measurement of the control of the pest. The results described are nevertheless of considerable interest. Dr Duddington is careful to point out the enormous gaps in our knowledge of the behaviour of predacious fungi under field conditions, and the difficulties involved in devising experiments to support the hypotheses which he puts forward.

AUDREY M. SHEPHERD

THE BRITISH MYCOLOGICAL SOCIETY TRANSACTIONS

Vol. 1 (part 3), 2 (1), 3 (2), 6 (3, 4), 7 (1-2, 3, 4), 8, (1-2, 3), 9 (1-2), 10 (1-2, 3, 4), 12 (1, 4), 16 (4), 17 (3), 18 (3, 4), 19 (2, 3, 4), 20 (1, 2), 21 (1-2), 22, (1-2), 23 (1, 2, 3, 4), 24 (1, 2, 3-4), 25 (1, 2, 3, 4), 26 (1-2, 3-4) 27 (1-2, 3-4), 29 (1-2, 3, 4). Single parts 10s., double parts 20s.; 30 (Jubilee Vol.), 30s.; Index, Vols. 1-30, 21s.; [31 (1-2), 33 (1-2, 3-4). Single parts 15s., double parts 30s.]*; 34-40, 60s. per volume. Postage extra.

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